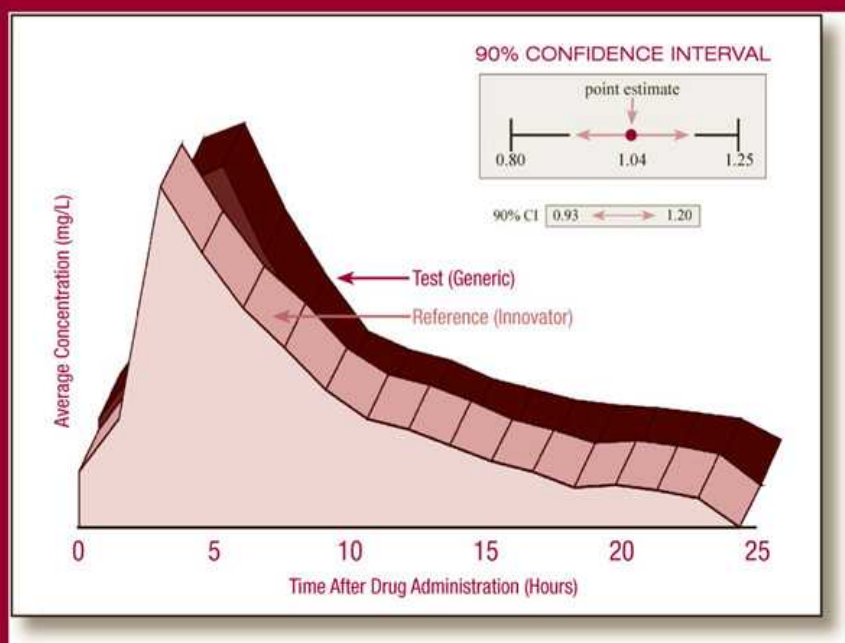


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Generic Drug Product Development Bioequivalence Issues



Edited by

Isadore Kanfer

Leon Shargel

**Generic Drug
Product Development
Bioequivalence Issues**

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Generic Drug Product Development Bioequivalence Issues

Edited by

Isadore Kanfer
Rhodes University
Grahamstown, South Africa

Leon Shargel
Applied Biopharmaceutics
Raleigh, North Carolina, USA

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healthcare

New York London

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Preface

This book is the second volume in a series of books on Generic Drug Product Development. The objective of the first book, *Generic Drug Product Development – Solid Oral Dosage Forms*, was to describe from concept to market approval the development of therapeutic equivalent generic drug products, including regulatory and legal challenges. This second volume, *Generic Drug Product Development – Bioequivalence Issues* focuses on current problems concerning the scientific demonstration of bioequivalence of two drug products.

Bioequivalence studies are very expensive, time consuming, and always have the possibility of failure. Failure to demonstrate bioequivalence of a proposed generic drug product results not only in a loss of money and time, but also may lead to a management decision not to pursue further development of this product.

Bioequivalence can be established for a large number of oral drug products that are intended for systemic drug absorption in which the drug and/or metabolites can be measured in biological fluid such as blood, plasma, serum, etc. For these drug products, the worldwide regulatory agencies and the scientific community are in agreement as to the design of a bioequivalence study and the statistical analyses of the results. For many other drug products, such as drugs intended for locally acting effects, highly variable drugs, and drugs with long elimination half-life bioequivalence can be very difficult to demonstrate.

Methods for the assessment of the bioequivalence of oral drug products that are intended for systemic drug absorption are well-documented and the approaches for such studies are described in guidances issued by many regulatory authorities throughout the world. While in general, the bioequivalence requirements of most regulatory bodies have much in common, in various instances specific issues and approaches may differ.

The objective of this volume is to discuss and explore various approaches for the demonstration of bioequivalence of drug products in which the regulatory agencies and the scientific community are not in agreement. These are usually related to drug products that have biopharmaceutical, bioavailability, pharmacokinetic, and pharmacodynamic properties that preclude the use of standard approaches that are outlined in published regulatory guidelines. The chapters in this volume

address those largely unresolved bioequivalence issues for the specific purpose of establishing therapeutically equivalent multisource (generic) drug products which will lead to regulatory approval and which can be confidently substituted for their brand-name counterparts.

Chapter 1 provides an introduction to the scientific principles underlying the assessment of bioequivalence, including various relevant definitions. The application of bioequivalence methodology and the approaches used to assess bioequivalence including statistical considerations and acceptance criteria are discussed.

The official position of the United States Food and Drug Administration, relevant to bioequivalence and therapeutic equivalence, is emphasized in Chapter 2. Approval of a generic drug product implies that such a product is a therapeutic equivalent to the brand product and may be safely substituted. This chapter will assist the reader in understanding the Food and Drug Administration position and what is required for generic drug approval.

Chapter 3 discusses pharmaceutical alternatives such as different salts and/or different dosage forms (e.g., capsule or tablet) that contain the same active pharmaceutical ingredient. This chapter examines whether pharmaceutical alternatives can be considered as therapeutic equivalents and interchangeable.

The use of pharmacodynamic measurements in lieu of plasma drug concentrations to assess bioequivalence is discussed in Chapter 4. The chapter discusses how the Emax model is used to relate changes in the pharmacodynamic response to changes in drug bioavailability.

The determination of bioequivalence using clinical endpoints is discussed in Chapter 5. Clinical endpoint bioequivalence studies are often used for locally acting drug products that are not intended for systemic absorption. Examples include topical anti-infective drugs, drugs given by inhalation, orally administered, non-absorbed drugs, ophthalmic, and otic drug products. The design and assessment of bioequivalence using clinical outcomes is also discussed.

Chapter 6 presents an overview of statistical considerations including alternate designs and approaches for bioequivalence assessments. Parallel study designs such as those needed for drugs with very long half-lives where a crossover study may be impractical are discussed as are the issues of outliers, studies performed in groups, and interim analyses. The vexing problem of the evaluation of highly variable drugs is discussed in Chapter 7. The problem of assessing the bioequivalence of these products and the implications of the usual regulatory conditions together with proposed solutions to resolve these issues are presented. The scaled average bioequivalence approach for highly variable drug products is presented together with the necessary computational procedures, limits and metrics, and associated statistical issues and recommendations.

Chapter 8 provides readers with a comprehensive account of population pharmacokinetic approaches to assessing bioequivalence, which includes compartmental versus non-compartmental pharmacokinetic approaches for bioequivalence. Mixed-effect modelling such as NONMEM and ITS2 are discussed and the advantages and disadvantages of the various methods and approaches are presented using case studies.

The role of metabolites in bioequivalence assessment is examined in Chapter 9. Presently, there is a lack of regulatory harmony regarding whether to monitor the parent drug and/or metabolite(s). An account of the formation of metabolites and associated implications for the assessment of bioequivalence is also provided.

Chirality and stereochemical considerations in bioequivalence are discussed in Chapter 10. This chapter provides a useful background with relevant definitions and associated terms. Reference is made to regulatory guidelines of the U.S.A., Canada, Europe, and Japan, and the limitations of these guidelines with respect to the implications of chirality for the assessment of bioequivalence are discussed. The effect of stereoselectivity on the pharmacodynamics and pharmacokinetics of chiral drugs and their formulation is discussed along with analytical methodology.

Food, including the quality and quantity, has been known to affect drug bioavailability, but not always in a predictable manner. It is sometimes not clear when to undertake a food effect bioequivalence study. Chapter 11 examines the effect of food on bioavailability and the use of a food-effect study in the assessment of bioequivalence.

The final Chapter, 12, discusses the role of endogenous drug substances in the determination of bioequivalence of drug products containing drugs that also occur naturally in the body. Potassium chloride and progesterone are used as examples. The chapter describes the pharmacokinetic and statistical assessment of endogenous substances administered exogenously including approaches in determining the endogenous drug concentration baseline and the factors affecting baseline stability.

The audience for this book includes undergraduate and graduate pharmacy students, pharmacy faculty, and drug manufacturers and regulators in the pharmaceutical industry who are interested in generic drug development and need more information concerning the current issues in bioequivalence assessment. The book discusses specific unresolved issues that are troubling to the scientific community and regulatory agencies and provides information on how to deal with such problems. Emphasis is on practical information for the development of protocols and the design and conduct of studies for the assessment of bioequivalence of generic drug products.

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Contributors

Sanford Bolton University of Arizona, Tucson, Arizona, U.S.A.

Charles Bon Biostudy Solutions LLC, Wilmington, North Carolina, U.S.A.

Philippe Colucci Faculté de Pharmacie, University of Montreal, Montreal, Quebec, Canada

Kim Dalhoff Clinical Pharmacologic Unit, Bispebjerg Hospital, Copenhagen, Denmark

Murray P. Ducharme Cetero Research, Cary, North Carolina, U.S.A. and Faculté de Pharmacie, University of Montreal, Montreal, Quebec, Canada

Laszlo Endrenyi Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada

Loren Gelber RRI Consulting Inc., Lake Wylie, South Carolina, U.S.A.

Christopher Hendy Novum Pharmaceutical Research Services, Pittsburgh, Pennsylvania, U.S.A.

Manish Issar Watson Laboratories, Inc., Corona, California, U.S.A.

André Jackson Food and Drug Administration, Office of Clinical Pharmacology, Silver Spring, Maryland, U.S.A.

Fakhreddin Jamali Faculty of Pharmacy, University of Alberta, Edmonton, Alberta, Canada

Isadore Kanfer Faculty of Pharmacy, Rhodes University, Grahamstown, South Africa

Jean-Francois Marier Pharsight Corporation, Mountain View, California, U.S.A.

Reza Mehvar School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, Texas, U.S.A.

Marika Pasternyk-Di Marco Pharmacokinetics and Pharmacodynamics, MDS Pharma Services, Montreal, Quebec, Canada

Diane Potvin Theratechnologies, Inc., Montreal, Quebec, Canada

Leon Shargel Applied Biopharmaceutics, Raleigh, North Carolina, and
Department of Pharmaceutics, School of Pharmacy, Virginia
Commonwealth University, Richmond, Virginia, U.S.A.

Jeffrey G. Stark Cedra Corporation, Austin, Texas, U.S.A.

Laszlo Tothfalusi Department of Pharmacodynamics, Semmelweis
University, Budapest, Hungary

Roger K. Verbeeck School of Pharmacy, Université Catholique de
Louvain, Brussels, Belgium

Roderick B. Walker Faculty of Pharmacy, Rhodes University,
Grahamstown, South Africa

Introduction—Bioequivalence Issues

Isadore Kanfer

*Faculty of Pharmacy, Rhodes University,
Grahamstown, South Africa*

Leon Shargel

*Applied Biopharmaceutics, Raleigh, North Carolina, and Department of
Pharmaceutics, School of Pharmacy, Virginia Commonwealth University,
Richmond, Virginia, U.S.A.*

GENERIC DRUG PRODUCT SUBSTITUTION AND THERAPEUTIC EQUIVALENCE

Generic drug products, also referred to as multi-source medicines, are drug products containing the same active pharmaceutical drug ingredient (API) in the same dosage form as that marketed by the innovator (brand) company. Generic drug products that meet national regulatory requirements for therapeutic equivalence and are approved by a regulatory agency can be substituted for their brand name counterparts with the full expectation that the generic drug product will produce the same clinical effect and safety profile as the prescribed product.

In the United States as in many other countries, a generic drug product is considered a *therapeutic equivalent* to the innovator (brand) drug product if it meets the regulatory requirements for therapeutic equivalence. To be classified as a therapeutic equivalent, generic drug products must meet the following general criteria as defined in the Orange Book (1):

1. They are approved as safe and effective
2. They are pharmaceutical equivalents in that they
 - (a) contain identical amounts of the same API in the same dosage form and are intended to be administered by the same route of administration, and

- (b) meet compendial or other applicable standards of strength, quality, purity, and identity and exert essentially the same effects with respect to both efficacy and safety.
- 3. They are bioequivalent in that
 - (a) they do not present a known or potential bioequivalence problem, and they meet an acceptable in vitro standard, or
 - (b) if they do present such a known or potential problem, they are shown to meet an appropriate bioequivalence standard.
- 4. They are adequately labeled
- 5. They are manufactured in compliance with Current Good Manufacturing Practice regulations.

The United States Food and Drug Administration (FDA) considers drug products to be therapeutically equivalent if they meet the criteria outlined above, even though they may differ in certain other characteristics such as shape, scoring configuration, packaging, excipients (including colors, flavors, preservatives), expiration date/time and minor aspects of labeling (e.g., the presence of specific pharmacokinetic information) and storage

It should, however, be emphasized that the concept of therapeutic equivalence does not encompass a comparison of different therapeutic agents used for the same condition (e.g., propoxyphene hydrochloride *vs.* pentazocine hydrochloride for the treatment of pain). Whereas initially bioequivalence was only applicable to pharmaceutically equivalent products, in some countries this is no longer a requirement (2–5). In these countries, products that are not pharmaceutical equivalents as defined by the Orange Book, may be considered therapeutic equivalents, and as such, substitutable. In those countries, therapeutic equivalence has been extended to include pharmaceutical alternatives. According to the European Agency for the Evaluation of Medicinal Products (2), “Medicinal products are pharmaceutical alternatives if they contain the same active moiety but differ in chemical form (salt, ester, etc.) of that moiety or in the dosage form or strength.” Furthermore, the term, pharmaceutical alternatives has also been included in their definition of bioequivalence, viz. “Two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same.” Similarly, WHO (3) has defined pharmaceutical alternatives as: “Products are pharmaceutical alternative(s) if they contain the same molar amount of the same active pharmaceutical moiety(s) but differ in dosage form (e.g., tablets versus capsules), and/or chemical form (e.g., different salts, different esters).” Pharmaceutical alternatives deliver the same active moiety by the same route of administration but are otherwise not pharmaceutically equivalent. They may or may not be bioequivalent or therapeutically equivalent with the comparator product.

According to the FDA (4), drug products are considered pharmaceutical alternatives if they contain the same therapeutic moiety, but are different salts, esters, or complexes of that moiety, or are different dosage forms or strengths (e.g., tetracycline hydrochloride, 250 mg capsules vs. tetracycline phosphate complex, 250 mg capsules, 250 mg capsules; quinidine sulfate, 200 mg tablets vs. quinidine sulfate, 200 mg capsules). In addition, FDA considers different dosage forms and strengths within a product line by a single manufacturer as pharmaceutical alternatives, as are extended-release products when compared with immediate-release or standard-release formulations of the same active ingredient. However, FDA does not at this time consider tablet and capsule formulations as a therapeutic equivalents even if they have been shown to be bioequivalent.

The reader is referred to Chapter 3 in this book that is devoted to a comprehensive discussion on pharmaceutical alternatives.

BIOAVAILABILITY

Bioavailability is defined as the *rate* and *extent* to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action (1). Bioavailability studies are important part of new drug development to establish a *systemic exposure* profile obtained by measuring the concentration of drug and/or metabolite concentration in the systemic circulation over time (4). Bioavailability data provides an estimate of the fraction of drug absorbed as well as drug distribution and elimination. Bioavailability studies are also used to develop a therapeutic dosage regimen. In generic drug development, bioequivalence studies are used to determine bioequivalence.

The most common approach for the determination of bioavailability is the *in vivo* measurement of active moiety or moieties in biologic fluid (e.g., plasma, urine). After a drug product is administered to a volunteer or patient, a plasma drug concentration curve versus time profile is obtained (Fig. 1). The major parameters representing the rate and extent of drug absorption are:

1. $C_{\max}$. The peak plasma drug concentration, $C_{\max}$ is used as a measurement for the rate of drug bioavailability. $C_{\max}$ has the units of mass/volume.
2. AUC. The area under the plasma level–time curve, AUC, is a measurement of the extent of drug bioavailability.
3. $T_{\max}$. The time of peak plasma concentration, $T_{\max}$, corresponds to the time required to reach maximum drug concentration after drug administration.

Based on the above, the rate ($C_{\max}$) and extent (AUC) to which the active ingredient is absorbed and becomes available from similar

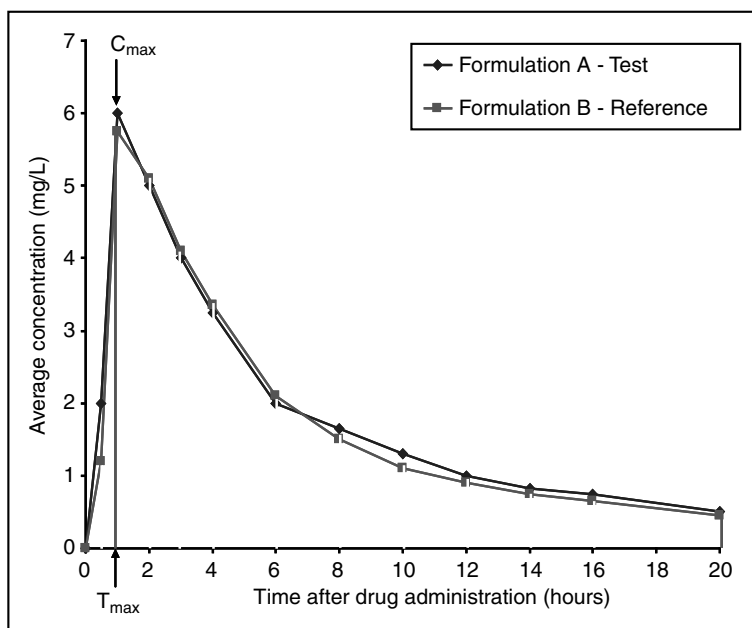


Figure 1 Bioequivalence of test and reference drug products.

formulations can readily be compared. As such these variables are thus used as surrogate measures of safety ($C_{\max}$) and efficacy (AUC). Hence, when different formulations of the same drug in identical dosage forms are compared on the basis of their bioavailabilities, and are found to be bioequivalent, it can reasonably be expected that they will exhibit essentially similar therapeutic and safety/toxicity profiles following administration to patients. It is, however, important that such products should be neither better nor worse relative to each other since they can only be equivalent or inequivalent.

For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action. Other methods for assessing bioavailability use endpoints such as acute pharmacodynamic effect, clinical observations or in vitro studies.

BIOEQUIVALENCE

Bioequivalent drug products are pharmaceutical equivalent or pharmaceutical alternative products that display comparable bioavailability to a reference drug product when studied under similar experimental conditions (1). The test drug product (usually the generic product) is considered

bioequivalent to the reference drug product (usually the brand product) if the rate and extent of absorption of the test drug does not show a significant difference from the rate and extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions. Both bioequivalence and bioavailability studies focus on the release of a drug substance from a drug product and subsequent absorption into the systemic circulation. For this reason, similar approaches to measuring bioavailability are used to assess bioequivalence.

The objective of a bioequivalence study is to compare formulation performance, between two or more drug products and is demonstrated by equivalent bioavailability.

Formulation performance is defined as the release of the drug substance from the drug product leading to bioavailability of the drug substance and eventually leading to one or more pharmacologic effects, both desirable and undesirable (6). Bioequivalence is a measure of formulation performance. After a drug product has been approved for marketing, a major change in the formulation (e.g., postapproval change) that affects components and composition, scale-up, site change, and manufacturing process or equipment changes may require an *in vivo* bioequivalence study (7,8). The postapproval bioequivalence study is necessary to demonstrate that the new formulation, manufacturing process, etc. and the performance of the new drug product is not different than the performance or the original drug product. Bioequivalence studies may also be used as “bridging” studies during new drug development when the innovator drug manufacturer changes the original clinical formulation of the drug used in the safety and efficacy studies to the final formulation to be marketed as the drug product.

Table 1 Approaches to Determining Bioequivalence

Method	Example
In vivo measurement of active moiety or moieties in biologic fluid (e.g., plasma, urine)	Oral drug products intended for systemic drug absorption
In vivo pharmacodynamic comparison	FEV ₁ —Albuterol Blanching Study—Topical Corticosteroids
In vivo limited clinical comparison	Nonsteroidal and other topical drug products not intended for absorption into the systemic circulation
In vitro comparisons	Comparative dissolution profiles
Any other approach deemed appropriate by FDA	Cholestyramine binding to bile acids

Various approaches for the determination of bioequivalence are listed in [Table 1](#). The measurement of the active drug in a biologic fluid is the most direct approach and generally has the least variability. The use of clinical endpoints to establish bioequivalence is generally the most variable in vivo approach and is least sensitive to small changes in drug bioavailability (see Chapter 5).

Bioequivalence may sometimes be demonstrated using an in vitro bioequivalence standard, especially when such an in vitro test has been correlated with human in vivo bioavailability data. In other situations, bioequivalence may sometimes be demonstrated through comparative clinical trials or pharmacodynamic studies. Where these above methods are not applicable (e.g., for drug products that are not intended to be absorbed into the bloodstream), other in vivo or in vitro test methods to demonstrate bioequivalence may be appropriate.

STATISTICAL CRITERIA FOR BIOEQUIVALENCE

Since different products containing the same active ingredient, or even the same product administered to the same subject on two separate occasions, rarely exhibit completely identical and superimposable profiles, some degree of difference must be considered acceptable to assure safety and efficacy without compromising therapeutic performance. Although, the substitution of a bioequivalent product necessitates that the therapeutic outcomes must be the same, hence sound scientific decision rules must be used for the declaration of bioequivalence. Such rules have been formulated using appropriate statistical criteria.

Study Designs

The most common design for a bioequivalence study that compares the test product to the reference product is a conventional nonreplicated design, such as the standard two-formulation, two-period, two-sequence crossover design. The crossover study is used most frequently for estimating bioequivalence of two or more drug products. The bioequivalence study is usually performed in a limited number of healthy volunteers, over 18 years of age or older and capable of giving informed consent. Generally, bioequivalence studies are conducted in subjects representative of the general population, taking into account age, sex, and race. Other subject populations may be used due to safety considerations. The assumptions in a crossover study are that drug clearance, volume of distribution, and absorption, as determined by physiological variables (e.g., gastric emptying, motility, pH), are assumed to have less inter-occasion variability compared to the variability arising from formulation performance. Therefore, differences between two products because of formulation factors can be

determined. Under certain circumstances, parallel designs can also be used (4). Other study designs may be used and are discussed in this book and elsewhere (9).

Reference Drug Product

FDA designates a reference-listed drug (RLD) as the standard to which all generic versions must be shown to be bioequivalent. The RLD is generally the brand-name drug that has been approved following the filing of a full new drug application. For US marketed drug products, the RLD is listed in the Orange Book (1). FDA hopes to avoid possible significant variations among generic drugs and their brand name counterpart. Such variations could result if generic drugs were compared to different reference drug products.

Statistical Considerations

The statistical methodology for analyzing these bioequivalence studies is called the two one-sided test procedure (9,10). Two situations are tested with this statistical methodology. The first of the two one-sided tests determines whether a generic product (test), when substituted for a brand-name product (reference) is significantly less bioavailable. The second of the two one-sided tests determines whether a brand-name product when substituted for a generic product is significantly less bioavailable.

Based on historical pharmacodynamic data and FDA medical experts, a significant difference in a clinical effect is not observed when a difference in plasma drug concentrations following administration of a test and reference product are less than 20%.

By convention, all bioequivalence data are expressed as a ratio of the average response (AUC and $C_{\max}$) for Test/Reference. The statistical criteria for acceptance of a generic product (test) are based on 90% confidence intervals (CIs) and not based upon differences in average values for AUC and $C_{\max}$. The 90% CIs for the Test product must fall within 80% to 120% of the Reference product based on nonlog transformed data. The use of log-transformed data tends to normalize the statistical distribution of the data. Therefore, based on log-transformed data, the 90% CIs for the test product must fall within 80% to 125% of the Reference product. The determination of the 90% CIs is performed by using two one-sided t -tests (9,10). These tests decide whether the response values (AUC and $C_{\max}$) for the test product is significantly greater or significantly less than those for the Reference product.

All data are thus log-transformed prior to conducting statistical testing. In practice, these statistical tests are carried out using an analysis of variance (ANOVA) procedure and calculating a 90% CI for each pharmacokinetic parameter ($C_{\max}$ and AUC). A 90% CI is used since it allows a 5% statistical error at both the upper and lower limits which translates into a 10% total

error, generating the 90% CI. The statistical procedure (“two one-sided test”) makes provision to accommodate “consumer risk” and “producer risk,” viz: Type I and Type II errors are typically set at 5%. Type I = declaration of bioequivalence for two products that are truly NOT bioequivalent (consumer risk) whereas Type II = declaration of nonequivalence for two products that are truly bioequivalent (producer risk).

Using these acceptance criteria, it would be difficult for any generic product whose mean arithmetic bioavailability parameters differ by more than 10% from the reference product to meet the CI requirements and clearly impossible to meet CI criteria if the differences approach 20%. In fact, a generic product that truly differs by -20% to $+25\%$ or more from the reference product with respect to either AUC and/or $C_{\max}$ would have less than a 5% chance of being accepted as bioequivalent.

The CI for both pharmacokinetic parameters, AUC and $C_{\max}$, must be entirely within the 80% to 125% boundaries cited above. Because the mean of the study data lies in the center of the 90% CI, the mean of the data is usually close to 100% (a Test/Reference ratio of 1). Different statistical criteria are sometimes used when bioequivalence is demonstrated through comparative clinical trials pharmacodynamic studies, or comparative in-vitro methodology. The bioequivalence methodology and criteria described above simultaneously control for both, differences in the average response between test and reference, as well as the precision with which the average response in the population is estimated. This precision depends on the within-subject (normal volunteer or patient) variability in the pharmacokinetic parameters (AUC and $C_{\max}$) of the two products and on the number of subjects in the study. The width of the 90% CI is a reflection in part of the within-subject variability of the test and reference products in the bioequivalence study. A test product with no differences in the average response when compared to the reference might still fail to pass the bioequivalence criteria if the variability of one or both products is high and the bioequivalence study has insufficient statistical power (i.e., insufficient number of subjects). Likewise, a test product with low variability may pass the bioequivalence criteria, when there are somewhat larger differences in the average response.

The results of a bioequivalence study are given in [Table 2](#). The parameters $C_{\max}$ and AUC are used as estimates of the rate and extent (bioavailability) of a test product compared to the reference product. In this case, the lower limit of the 90% CI for $C_{\max}$ is 76.5%, just outside the permissible CIs of 80% to 125%. Therefore, this test product fails the criteria for bioequivalence.

The results were obtained from a two-way, crossover, single dose study in 36 fasted healthy, adult male and female volunteers. No statistical differences were observed for the mean values between Test and Reference products. All results are based on ln-transformed data.

Table 2 Bioavailability Comparison of a Generic (Test) and Brand (Reference) Drug Product

PK variable	Geometric mean		Reference	%Ratio T/R	90% Confidence interval (lower limit, upper limit)	P-values for product effects	Power of ANOVA	ANOVA % CV
	Units	Test						
C _{max}	ng/mL	294.37	356.81	82.3	(76.5,112)	0.3586	0.8591	19.90%
AUC _{0-t}	ng hr/mL	2659.12	2674.92	99.4	(95.1,104)	0.8172	0.9876	12.60%
AUC _{int}	ng hr/mL	2708.63	2718.52	99.6	(95.4,103)	0.8865	0.9678	12.20%
T _{max}	hr	5.12	4.24	1.21				
K _{elim}	1/hr	0.0961	0.098	98.1				
t _{1/2}	hr	8.47	8.33	101.7				

Bioequivalence

As shown in Table 2 above, the test product failed the bioequivalence criteria for C_{max} . From the results, the generic drug manufacturer needs to know whether the test product was poorly formulated and truly bioequivalent to the reference product or whether the test product could have been shown to be bioequivalent to the reference product if the study were better designed.

Bioequivalence can be attributed to two possible study design problems. First, if the plasma sampling intervals were not optimally taken at the most appropriate time periods. This is often a problem with the C_{max} value that is the highest observed plasma drug concentration taken at T_{max} (Fig. 1). For immediate release, rapidly absorbed drug products, the peak drug concentration may be very sharp and the true T_{max} (thus the C_{max} value) may be missed. Second, the subjects may not have an optimal number of subjects enrolled in the study due to higher than expected intrasubject variability. This may be observed in the results by a low value for the power of the ANOVA. A pilot bioequivalence study can often help to determine the appropriate sampling times and from knowledge of the intrasubject variability, the correct number of subjects can be estimated for a pivotal bioequivalence study.

The data from six different bioequivalence studies are shown in Figure 2. The data in the two studies at the top in the figure meet the criteria for bioequivalence even though the data in the second from the top diagram

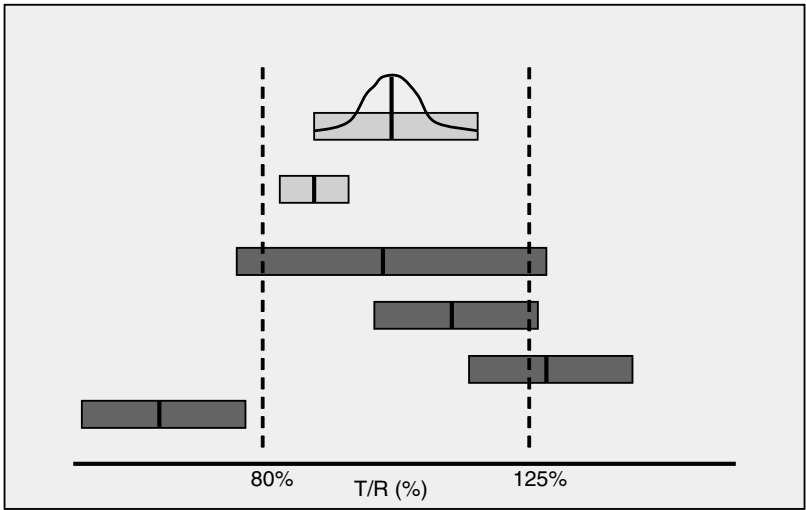


Figure 2 Possible bioequivalence results (90% confidence intervals). *Source:* Adapted from Ref. 6.

is skewed to the lower side of the 90% CI. The data in the next two studies (third and fourth diagrams from the top in Fig. 2) fail to meet the 90% CIs. Since the mean ratios are well within the 80% to 125% CIs, the products in these two studies show bioinequivalence. It is possible that the test product might demonstrate bioequivalence to the reference product if a better study design such as a larger subject population were used. The data in the two studies depicted in the bottom part of the figure show that the test product fails bioequivalence, since the mean Test/Reference ratios are outside the 80 to 125% range and much of the data are outside the lower or the upper ends.

BIOEQUIVALENCE ISSUES

Bioequivalence can readily be demonstrated for oral drug products that contain drugs that are well absorbed systemically as the intact drug and have low intrasubject variability (less than 30% CV). For many drug products, such as drugs that show high intrasubject variability (see Chapter 7) and locally acting drugs, the determination and establishment of bioequivalence is more challenging.

Table 3 summarizes many of the issues that complicate the determination of bioequivalence of two drug products. Some of these bioequivalence issues will be discussed separately in this volume and subsequent volumes. However, apart from the general issues relating to the assessment of bioequivalence based on the availability of guidance documents, several other issues not specifically addressed present further challenges to sponsors.

The *modus operandi* of the process and related acceptance criteria have evolved since its inception and has certainly served the generic pharmaceutical industry extremely well over the years. Although the conduct of BE studies has been well-documented in numerous regulatory bulletins, guidances and related publications and the acceptance criteria have been well-established, some

Table 3 Bioequivalence Issues

Pharmaceutical alternatives
Drug analysis in biological fluids and/or pharmacodynamic or clinical studies?
Highly variable drugs
Moieties to be measured?
–Parent drug versus metabolites
–Enantiomers versus racemates
Endogenous drug substances
Effect of food
Long half-life drugs
First point $C_{\max}$
In vitro studies as a surrogate for bioequivalence?

differences exist in the regulatory requirements in different countries around the world. In most cases these differences are relatively minor, however, certain specific issues remain controversial and unresolved.

Table 3 summarizes some of the issues that complicate the determination of bioequivalence of two drug products. The chapters that follow have been included to provide the current thinking and approaches which may be followed in order to address the various issues which remain largely unresolved and the questions that remain largely unanswered.

Pharmaceutical Alternatives

Can a tablet be compared with a capsule dosage form or products which contain the same chemical moiety in a different salt/ester form be substituted if found to be bioequivalent? Can products containing the same API and found to be bioequivalent even when administered by different routes be substituted?

Nonoral Drug Products and Drug Products Intended for Local Activity

The determination of bioequivalence for a variety of nonoral drug products and drug products intended for local activity can be difficult. The bioequivalence of a true aqueous drug solution is often not required because bioequivalence is self-evident. As drug products become more complicated, such as modified release drug products, parenteral drug products that are suspensions or formulated in liposomes, locally active drug products such as inhalation, ocular, topical and ophthalmic products, bioequivalence of such products becomes more difficult to demonstrate. For these products, clinical or pharmacodynamic endpoints may be needed.

Highly Variable Drugs

Meeting bioequivalence requirements are often very difficult for drugs that are highly variable, generally those drugs with 30% or greater intrasubject coefficient of variation. Due to high intrasubject variability, a large subject population ($n > 80$) is needed to meet the 90% confidence intervals of 80 to 125%. Can special measures and statistical approaches be used for such classes of drugs or special dispensations made by adjusting the acceptance criteria?

Moieties to be Measured

If the parent is a prodrug or is highly metabolized, should the parent drug and/or the metabolites be measured? The physical-chemical and pharmacokinetic properties of the active pharmaceutical ingredient(s) or API may influence the determination of bioequivalence. For drugs that exhibit chirality, should enantiomers or racemates be measured? Some drugs have very long biological half-lives. How long should the drug be monitored and measured?

Endogenous Substances Used as Drugs

Hormonal drugs may be structurally the same as endogenous drug substances. Should the analytical measurements for the API be adjusted for baseline concentrations of endogenous substances?

Food Effect Studies

Administration of a drug product with food may change the bioavailability by affecting either the drug substance or the drug product. Food effect bioequivalence studies are conducted to assess the effects of food on the rate and extent of absorption of a drug when the test drug product or the reference drug product is administered shortly after a meal (fed conditions)(8). Food effects on bioavailability are generally greatest when the drug product is administered shortly after a meal is ingested. The nutrient and caloric contents of the meal, the meal volume, and the meal temperature can cause physiological changes in the gastrointestinal tract in a way that affects drug product transit time, luminal dissolution, drug permeability, and systemic availability. In general, meals that are high in total calories and fat content are more likely to affect the gastrointestinal physiology and thereby result in a larger effect on the bioavailability of a drug substance or drug product.

Narrow Therapeutic Index Drugs

Narrow therapeutic index (NTI) drugs are potent drugs, such as digoxin, lithium, phenytoin and warfarin that require therapeutic drug monitoring. Small changes in plasma drug concentrations can have a large change in pharmacodynamic activity. Are special measures necessary to assess the bioequivalence of drugs that have a narrow therapeutic index drugs?

SUMMARY

The assessment of bioequivalence provides a valuable tool to establish the comparable safety and efficacy of generic drug products. The demonstration of bioequivalence is often the most difficult problem and possibly the most expensive part in the development of a generic drug product. Factors affecting bioavailability include the physical-chemical behavior of the active drug, the pharmacokinetics of the drug, the type of dosage form, the route of drug administration and the intended therapeutic effect (local or systemic activity).

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Approved Drug Products with Therapeutic Equivalence Evaluations (The Orange Book)

Loren Gelber

RRI Consulting Inc., Lake Wylie, South Carolina, U.S.A.

INTRODUCTION

It is entirely appropriate that a book about unresolved issues in bioequivalence testing starts with a chapter about the *Approved Drug Products with Therapeutic Equivalence Evaluations* (the Orange Book) (1). Since 1979, the U.S. Food and Drug Administration (FDA) has used the Orange Book to communicate its official position on which drug products are bioequivalent and which products are not. In keeping with the focus of this volume, this chapter will emphasize those aspects of the Orange Book that are most relevant to bioequivalence. The Orange Book also contains a great deal of other valuable information, which will be discussed briefly later in the chapter.

WHAT IS THE ORANGE BOOK?

The Orange Book is a book of lists. The most important section of the Orange Book for the purposes of this volume is a list of all prescription pharmaceutical products that have been approved for sale in the United States by the FDA. This includes products approved via a new drug application (NDA), abbreviated new drug application (ANDA) or biological license application (BLA). If the FDA has approved a product as a therapeutic equivalent of another product, both products will have therapeutic equivalence codes next to their names. These codes will be explained in this chapter.

A brief discussion of the history and background of the Orange Book is included in the preface to each yearly edition. In January 1979, FDA published a proposed list; the first official list was published in October 1980. The 27th edition was published in January 2007. It is called the Orange Book because someone in the Government Printing Office decided in 1979 or 1980 to put an orange cover on the book. Actually, it was rather slim, more like a pamphlet than a book. Over the years it has grown. The paper editions are now more than two inches thick. The 26th edition has 1022 pages. Since the official name of the book is rather long, it is generally called the Orange Book.

Today, the Orange Book is available as a portable document format (pdf) file and as a web site, called the Electronic Orange Book. The home page of the Electronic Orange Book is <http://www.fda.gov/cder/ob/default.htm>. One can access the pdf file from the home page. Annual subscriptions are available from the US Government Printing Office. There is also a newer web site, called Drugs@FDA (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Search_Drug_Name) that performs some of the same functions and offers some new information. These web sites will also be discussed in this chapter.

WHAT IS IN THE ORANGE BOOK?

Figure 1 is a copy of the table of contents of the 26th edition. It can be noted that the book contains quite a few pages of explanatory text in the beginning.

Introduction to the Orange Book

The Introduction starts with a general explanation of what is and what is not listed in the Orange Book. Those drugs that are marketed in the United States without an approved application are not in the Orange Book. This includes over-the-counter (OTC) drugs which do not require an approved application because they conform to one of the OTC monographs published by FDA in the Code of Federal Regulations (CFR) sections 328 to 358. However, OTC drugs marketed on the basis of an approved NDA or ANDA are listed, in the OTC Drug Product List.

As explained in the preface, there are also drugs on the market in the United States that without an application because they were marketed in the United States before 1938. An approved NDA, based on a showing of safety only, was first required based on the amendments to the Federal Food, Drug and Cosmetic Act (FFDCA) in 1938. This requirement was not made retroactive by Congress. FDA Officials have stated at various public forums that in order to qualify for the

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Therapeutic Equivalence Evaluations
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Figure 1 The table of contents of the 27th edition of the 2007 Orange Book.

“grandfather” status a product would have to unchanged in labeling (and possibly in formulation) since 1938 (2). The only example of a “grandfather” drug product given in the Preface to the 2006 Orange Book is phenobarbital tablets.

In 1962, Congress added a requirement that a drug be proved effective to the FFDCA. This started a long process of reviewing all drugs that had been approved between 1938 and 1962. The process is known as the Drug Efficacy Study Implementation (DESI) review. There are a few drug products that have not received a final determination as to their effectiveness; they are generally referred to as DESI drugs. The Preface to the 2006 Orange Book gives two examples, Donnatal[®] Tablets and Librax[®] Capsules. These drugs are also not listed in the Orange Book, because they do not have full, approved NDAs or ANDAs.

The Introduction to the Orange Book continues with an explanation of the terms “pharmaceutical equivalents,” “pharmaceutical alternatives,” “therapeutic equivalents,” “bioavailability” and “bioequivalent drug products.” Pharmaceutical equivalents are the same in active ingredient, route of administration dosage form and strength, but are not necessarily bioequivalent. Pharmaceutical alternatives contain the same therapeutic moiety, but may differ in counter-ion, ester, complex or dosage form. Thus a 100 mg tablet and a 100 mg capsule of the same active ingredient are pharmaceutical alternatives, even if bioequivalent. Therapeutic equivalents are pharmaceutical equivalents that are bioequivalent. They also have to meet all standards for quality, are properly labeled and manufactured according to the FDA’s Current Good Manufacturing Practice regulations.

Bioavailability is defined in the 1984 amendments to the FFDCA. It is the rate and extent to which the active ingredient or active moiety is absorbed from the drug product (for products intended to be absorbed into the blood stream) and becomes available at the site of action. The theoretical definition of bioequivalent is equally bioavailable. In practice establishing equal bioavailability can be extremely complicated. How to do so is the subject of the remainder of this volume. The Introduction to the Orange Book offers some definitions, a brief discussion of the types of studies that are needed to establish bioequivalence and a summary of the standard statistical methods used in these studies.

The next topic tackled in the Introduction to the Orange Book is the reference listed drug. The 1984 amendments to the FFDCA and current FDA regulations [21CFR 314.94 (a)(3)] require that a generic drug have an approved product to which it is declared to be equivalent. This approved product is called the reference-listed drug. In most cases it is the product of the innovator that first introduced the active ingredient to the U.S. marketplace via an NDA. The listings in the Orange Book indicate which product is to be used as a reference-listed drug.

The Introduction then includes some legal and regulatory information that is applicable to pharmacists who are responsible to decide on substitution of a generic product for the brand on the patient's prescription form. This topic is outside the scope of this chapter and will not be discussed here.

Therapeutic Equivalence Codes

The next section of the Orange Book is probably the most important one for readers of this volume. It explains the therapeutic equivalence codes, which FDA includes in the listing of approved, marketed prescription drug products. These codes consist of two letters, sometimes followed by a number. The first letter is either A or B. If the first letter is A, the products listed in that section of the Orange Book prescription product list have been declared by FDA to be therapeutically equivalent; if the first letter is B, they have not been determined to be therapeutically equivalent.

The FDA uses the second letter of the therapeutic equivalence code to make subdivisions within the A and B classifications.

If the therapeutic equivalence code is AA, this means that in vivo bioequivalence trials are not required to establish therapeutic equivalence. Oral dosage forms of AA products, other than solutions, must meet an appropriate in vitro dissolution test to receive an AA rating.

In contrast, if the code is AB, a bioequivalence trial of the type discussed in this volume is required to demonstrate therapeutic equivalence. In some cases there is more than one listed drug. This occurs when FDA has approved more than one pharmaceutical equivalent of a drug product but these products have not been demonstrated to be bioequivalent to one another. If some generics are shown to be equivalent to one of the alternatives while others are shown to be equivalent to a different alternative, the FDA adds a numeral to the end of the code so that these products can be distinguished from one another. Thus one may have a rating of AB1, AB2 etc. This situation is more likely to occur with sustained release products.

There are four other codes listed in the Orange Book that start with A. These codes cover special situations relevant to specific dosage forms. FDA assigns the AN code to solutions and powders intended to aerosolization and marketed for use in any of several delivery systems. This code is also assigned to solutions or suspensions in specific delivery systems if they are approved based on in vitro methodology, rather than a bioequivalence trial. The reason given in the Introduction to the Orange Book is that there is some uncertainty regarding the therapeutic equivalence of the products due to differences in the delivery systems. The AO code is used for injectable oil solutions where the type of oil in the generic differs from that in the innovator. FDA uses the AP code for injectable aqueous solutions and some intravenous solutions. The reason given is that some of these products differ from the reference-listed drug in route of administration, preservatives

or need for reconstitution. The AT code is used for solutions and DESI topical products were approved based on a waiver of bioequivalence requirements. These are products whose reference-listed drug was approved before 1962.

There are ten different therapeutic equivalence codes listed in the Introduction to the Orange Book that start with B. FDA has stated that the reference-listed drugs for the products approved with B codes were approved before the effective date of the 1984 amendments to the FDCA. (However, see an exception below.) These products are not bioequivalent to one another and are not supposed to be substituted by pharmacists.

The FDA uses the B* code if new information is received that raises a significant question regarding therapeutic equivalence, during the process of an investigation. This code was used in 1989 and 1990 and usually if not always resulted in the withdrawal of the product in question.

The BC code is used for extended-release capsule, injectables and tablets that are not demonstrated to be bioequivalent to one another. The BD code indicates that the products contain active ingredients with known bioequivalence problems that have not been demonstrated to be bioequivalent to one another. BE is a similar code for delayed-release oral dosage forms, BN for aerosol-nebulizer drug delivery systems and BR for suppositories or enemas that deliver drugs for systemic absorption.

In contrast to the dosage-form specific B codes, FDA has also defined and used other types of B codes. The BP code indicates that the two products contain active ingredients or are formulated in dosage forms with potential bioequivalence problems. This code has been used for pharmaceutically equivalent products until adequate in vivo bioequivalence data has been submitted, including injectable suspensions. The Introduction to the Orange Book states that if the drug standards for an active ingredient are found to be deficient, FDA will assign the BS code to all drug products that contain this active ingredient. The final code, BX, is assigned to products for which the data that has been reviewed by the agency is insufficient to prove bioequivalence, and thus therapeutic equivalence. One may conclude from this paragraph that if no data was submitted, the pre-1984 product got a BP rating, while if the data submitted did not meet FDA's standards, the product got a BX rating.

Additional Sections

There are several sections at the end of the Introduction to the Orange Book. One describes special situations for amino acid and protein hydrolysate injections, follitropin alfa and beta, Gaviscon[®] and levothyroxine sodium. In the case of levothyroxine sodium, there are three reference listed drugs that have therapeutic equivalents and are rated AB1, AB2 and AB3.

Two of the products have all three codes, because they have demonstrated bioequivalence to all the three reference drugs. Several others have two codes. Three more are coded BX as they have not been demonstrated to be bioequivalent to any other product. This creates a very complicated situation for the pharmacist who need to substitute any one of these products for another.

This discussion is followed by a paragraph about products that are approved based on a suitability petition and one about waived exclusivity, which are not relevant to this volume. The introduction ends with a discussion of the procedures FDA will use to change a therapeutic equivalence code and the way that discontinued products that have been determined by FDA not to have been withdrawn for safety and efficacy reason are annotated in the Discontinued Product list. The Introduction to the Orange Book then explains how to reach the Orange Book staff to notify them of needed changes and corrections. The Introduction ends with an explanation that the Orange Book is now an electronic document and how to order a subscription.

How to Use the Drug Product Lists

Chapter 2 of the Orange Book includes two pages of brief explanation and two illustrations. All of the items that can be found in an Orange Book listing are enumerated without explanation. The way products with more than one active ingredient are alphabetized is explained, as are the three tables that appear in the Appendix.

The two illustrations in Chapter 2 do not represent real products. They are annotated to help the reader understand how the Orange Book listings are arranged. The first illustration is shown in [Figure 2](#). A short explanation will attempt to clarify this illustration.

The top part of the illustration, labeled “Single Ingredient,” shows five different Meperidine Hydrochloride Injections. The reader can determine this as follows: the first line, underlined and left justified, is the name of the active ingredient, and the second line, indented and not underlined, is the dosage form and route of administration. On the third line, underlined and further indented, is the word “Hexanon.” This is the brand name or trade name of the product. The line under that gives an abbreviation of the name of the firm that owns the application for Hexanon. Four different strengths of Hexanon are listed next, each on its own line. Next the generic name “Meperidine HCl” appears on a separate line, underlined. It is followed by the names of four manufacturers of generic Meperidine HCl injections, thus we have five total products. (Donhare Pharm is an inside joke. Don Hare is the FDA employee most responsible for the existence and usefulness of the Orange Book.)

There is more information that can be gleaned from this listing, as the illustration demonstrates. The words “Hexanon” and “Meperidine HCL”

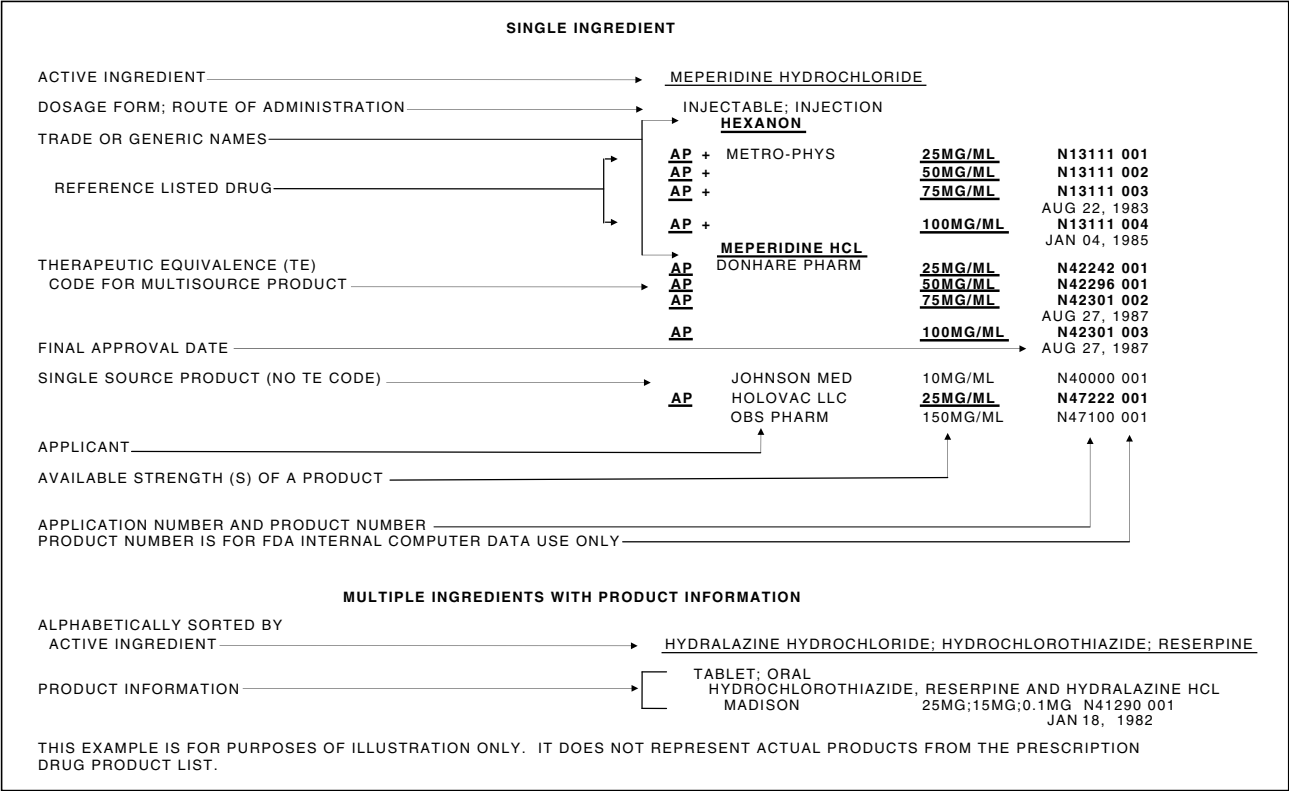


Figure 2 Illustration from Chapter 2 of the Orange Book.

are bold and underlined. FDA uses this bold underlining of the name and strength as one way that the reader can tell that a product has therapeutic equivalents. The brand manufactured by Metro-Phys and the generic manufactured by Donhare Pharm are both available in the same four strengths. At the beginning of each line listing these eight products is the therapeutic code “AP,” also underlined. This means that these products and strengths have been declared by FDA to be therapeutically equivalent to each other and a pharmacist may substitute one for the other.

There is a plus sign after the codes for Hexanon. This means that any applicant who wishes to file an ANDA for Meperidine HCl injection in the strengths available as Hexanon must use Hexanon as the reference listed drug. This designation is important when there is more than one brand of the same product, when the brand has been discontinued but generics remain on the market and other similar situations.

In this illustration, the manufacturer Holovac LLC is approved for only one of the strengths available as Hexanon. There is an “AP” code on this line and the strength is underlined, allowing the reader to determine that this Holovac product is therapeutically equivalent to the Metro-phys and Donhare products. The other two manufacturers have approval for strengths that are unique. Therefore they have no codes next to their listings and their strengths are not underlined. Since two products must be of the same strength to be therapeutically equivalent, these two unique strengths are not so designated.

The right hand column of the listing gives application numbers and approval dates. In the illustration shown in [Figure 2](#), three of the Hexanon strengths were approved in the same application on the same date. The approval date shown after product number 003 applies to all three strengths above it. An additional strength was approved later; its approval date is listed after it. All four generics from Donhare Pharm were approved on the same date but they have three different application numbers.

It should be noted that application numbers are listed in the Orange Book in a slightly different format than that used in official FDA correspondence. They are listed with the letter N in front and without the usual hyphen. Thus the first NDA number listed in [Figure 2](#), N13111, would usually be written 13-111. This fact becomes a bit more important when we discuss [Drugs@FDA](#) below.

The second part of the illustration shows how products that contain more than one active ingredient are listed in the Orange Book. The active ingredients will be listed on the first line of the entry in alphabetical order. The product name will appear after the dosage form line with the ingredients in the order they appear in the name of the product approved by FDA in the application.

The second illustration in Chapter 2 of the Orange Book serves to emphasize that two products rated AB are considered therapeutically

27TH EDITION - 2007 - APPROVED DRUG PRODUCTS LIST					
PRESCRIPTION DRUG PRODUCT LIST				3 - 1(of370)	
<u>ABACAVIR SULFATE</u>					
SOLUTION; ORAL					
ZIAGEN					
+ GLAXOSMI THKLINE		EQ 20MG BASE/ML	N20978	001	Dec 17, 1998
TABLET; ORAL					
ZIAGEN					
+ GLAXOSMI THKLINE		EQ 300MG BASE	N20977	001	Dec 17, 1998
<u>ABACAVIR SULFATE; LAMIVUDINE</u>					
TABLET; ORAL					
EPZICOM					
+ SMITHKLINE BEECHAM		EQ 600MG BASE; 300MG	N21652	001	Aug 02, 2004
<u>ABACAVIR SULFATE; LAMIVUDINE; ZIDOVUDINE</u>					
TABLET; ORAL					
TRIZIVIR					
+ GLAXOSMI THKLINE		EQ 300MG BASE;150MG; 300MG	N21205	001	Nov 14, 2000
<u>ACAMPROSATE CALCIUM</u>					
TABLET, DELAYED RELEASE; ORAL					
CAMPRAL					
+ FOREST LABS		333MG	N21431	001	Jul 29, 2004
<u>ACARBOSE</u>					
TABLET; ORAL					
PRECOSE					
BAYER PHARMS		25MG	N20482	004	May 29, 1997
		50MG	N20482	001	Sep 06, 1995
+		100MG	N20482	002	Sep 06, 1995
<u>ACEBUTOLOL HYDROCHLORIDE</u>					
CAPSULE; ORAL					
<u>ACEBUTOLOL HYDROCHLORIDE</u>					
<u>AB</u>	ALPHAPHARM	<u>EQ 200MG BASE</u>	<u>N75047</u>	<u>001</u>	Dec 30, 1999
<u>AB</u>		<u>EQ 400MG BASE</u>	<u>N75047</u>	<u>002</u>	Dec 30, 1999
<u>AB</u>	MYLAN	<u>EQ 200MG BASE</u>	<u>N74288</u>	<u>001</u>	Apr 24, 1995
<u>AB</u>		<u>EQ 400MG BASE</u>	<u>N74288</u>	<u>002</u>	Apr 24, 1995
<u>AB</u>	WATSON LABS	<u>EQ 200MG BASE</u>	<u>N74007</u>	<u>001</u>	Oct 18, 1995
<u>AB</u>		<u>EQ 400MG BASE</u>	<u>N74007</u>	<u>002</u>	Oct 18, 1995
<u>SECTRAL</u>					
<u>AB</u>	DR REDDYS LABS INC	<u>EQ 200MG BASE</u>	<u>N18917</u>	<u>001</u>	Dec 28, 1984
<u>AB</u>	+	<u>EQ 400MG BASE</u>	<u>N18917</u>	<u>003</u>	Dec 28, 1984
<u>ACETAMINOPHEN; ASPIRIN; CODEINE PHOSPHATE</u>					
CAPSULE; ORAL					
ACETAMINOPHEN, ASPIRIN, AND CODEINE PHOSPHATE					
+ MIKART		150MG;180MG;30MG	N81096	001	Oct 26, 1990
<u>ACETAMINOPHEN; BUTALBITAL</u>					
CAPSULE; ORAL					
<u>BUCET</u>					
<u>AB</u>	MALLINCKRODT	<u>650MG;50MG</u>	<u>N88991</u>	<u>001</u>	Jun 28, 1985
<u>PHRENILIN FORTE</u>					
<u>AB</u>	+ VALEANT	<u>650MG;50MG</u>	<u>N88831</u>	<u>001</u>	Jun 19, 1985
<u>TENCON</u>					
<u>AB</u>	MALLINCKRODT	<u>650MG;50MG</u>	<u>N89405</u>	<u>001</u>	May 15, 1990
TABLET; ORAL					
<u>BUTAPAP</u>					
<u>AB</u>	MIKART	<u>325MG;50MG</u>	<u>N89987</u>	<u>001</u>	Oct 26, 1992

Figure 3 First page of the approved prescription products list.

equivalent to each other while a product rated BP is considered not therapeutically equivalent to them.

List of Approved Prescription Drugs

We have finally reached the main list in the Orange Book, the list of approved prescription drugs. In the 2006 edition it is 360 pages long. A copy of the first page of this list is reproduced in [Figure 3](#). A consideration of some of the information on this page may help the reader reinforce the information given above on how to use this list.

The first product listed is abacavir sulfate. Only the brand Ziagen® is listed, as no therapeutic equivalents were approved by FDA as of January 1, 2006. It is available in two dosage forms, a solution and a tablet. There is a plus sign next to each product listing, indicating that they are to be used as reference listed drugs.

The first product listing in the 2006 Orange Book that has therapeutic equivalents is acebutolol hydrochloride. It is available as an oral capsule. The generics are listed first because acebutolol comes before Sectral® in the alphabet. From the listing the reader can determine that there are three manufacturers of acebutolol hydrochloride capsules that are rated by FDA as therapeutically equivalent to Sectral® and FDA has not been informed that any of these products was withdrawn from the market as of January 1, 2006. The reader can also determine that if another firm wishes to develop a generic acebutolol hydrochloride capsule, the bioequivalence trial must be done using Sectral® capsules equivalent to 400 mg of acebutolol base, because there is a plus sign on that line in the listing.

List of OTC Drugs

The first page of the OTC drug product list is reproduced in [Figure 4](#). When compared to Figure 3, there are many similarities and some differences. The most striking difference is the absence of therapeutic equivalence codes. FDA does not consider these codes relevant to OTC drugs, since the purpose of the codes is to guide pharmacists in substituting one product for another. However, the plus signs are present next to quite a few entries. This tells the potential applicant that in order to have a generic of one of these products approved, the applicant must demonstrate bioequivalence to the designated reference listed drug.

The reader is reminded that those OTC drugs which do not require an approved application because they conform to one of the OTC monographs published by FDA in the CFR sections 328 to 358 are not listed in the Orange Book List of OTC Drugs.

27 TH EDITION - 2007 - APPROVED DRUG PRODUCTS LIST				
OTC DRUG PRODUCT LIST				
4 - 1 (of 15)				
<u>ACETAMINOPHEN</u>				
SUPPOSITORY; RECTAL				
ACEPHEN				
G AND W LABS	120MG	N18060	001	
	120MG	N72218	001	Mar 27, 1992
	325MG	N18060	003	Dec 18, 1986
	325MG	N72344	001	Mar 27, 1992
	650MG	N18060	002	
	650MG	N72237	001	Mar 27, 1992
ACETAMINOPHEN				
ACTAVIS MID ATLANTIC	120MG	N18337	003	Sep 12, 1983
	325MG	N18337	002	
+	650MG	N18337	001	
SUPPOSITORIA	120MG	N70607	001	Apr 06, 1987
	650MG	N70608	001	Dec 01, 1986
INFANTS' FEVERALL				
ACTAVIS MID ATLANTIC	80MG	N18337	004	Aug 26, 1992
NEOPAP				
POLYMEDICA	120MG	N16401	001	
TABLET, EXTENDED RELEASE; ORAL				
ACETAMINOPHEN				
COREPHARMA	650MG	N76200	001	Mar 19, 2002
PERRIGO	650MG	N75077	001	Feb 25, 2000
TYLENOL (CAPLET)				
+ MCNEIL CONS	650MG	N19872	001	Jun 08, 1994
TYLENOL (GELTAB)				
+ MCNEIL CONS	650MG	N19872	002	Jan 11, 2001
<u>ACETAMINOPHEN; ASPIRIN; CAFFEINE</u>				
TABLET; ORAL				
ACETAMINOPHEN, ASPIRIN AND CAFFEINE				
PERRIGO	250MG;250MG;65MG	N75794	001	Nov 26, 2001
EXCEDRIN (MIGRAINE)				
+ NOVARTIS	250MG;250MG;65MG	N20802	001	Jan 14, 1998
<u>ACETAMINOPHEN; CLEMASTINE FUMARATE; PSEUDOEPHEDRINE HYDROCHLORIDE</u>				
TABLET; ORAL				
TAVIST ALLERGY/SINUS/HEADACHE				
+ NOVARTIS	500MG;EQ 0.25MG BASE;30MG	N21082	001	Mar 01, 2001
<u>ACETAMINOPHEN; DEXBROMPHENIRAMINE MALEATE; PSEUDOEPHEDRINE SULFATE</u>				
TABLET, EXTENDED RELEASE; ORAL				
DRIXORAL PLUS				
+ SCHERING PLOUGH	500MG;3MG;60MG	N19453	001	May 22, 1987
<u>ALCOHOL; CHLORHEXIDINE GLUCONATE</u>				
SOLUTION; TOPICAL				
AVAGARD				
+ 3M	61%;1%	N21074	001	Jun 07, 2001
<u>ALUMINUM HYDROXIDE; MAGNESIUM TRISILICATE</u>				
TABLET, CHEWABLE; ORAL				
FOAMCOAT				
GUARDIAN DRU	80MG;20MG	N71793	001	Sep 04, 1987
GAVISCON				
SANOFI AVENTIS US	80MG;20MG	N18685	001	Dec 09, 1983
+	160MG;40MG	N18685	002	Dec 09, 1983
<u>ANTAZOLINE PHOSPHATE; NAPHAZOLINE HYDROCHLORIDE</u>				
SOLUTION/DROPS; OPHTHALMIC				
VASOCON-A				
+ NOVARTIS	0.5%;0.05%	N18746	002	Jul 11, 1994

Figure 4 First page of the OTC drug product list.

Discontinued Drug Product List

The Discontinued Drug Product list is organized in a manner to the prescription and OTC lists. It lists products that have been approved by FDA but the owner of the product has informed FDA that they are not currently being marketed. Since they are not on the market they do not have any therapeutic equivalence codes.

One of the most useful aspects of this list is a notation that can be found in the listing of certain products. The notation reads “Federal Register determination that was not discontinued or withdrawn for safety or efficacy reasons.” This means that someone petitioned the FDA to make this determination, probably because there was some interest in bringing it back to the marketplace. This FDA determination is required before FDA will accept an application for a product on this list. Products that are withdrawn when there is no problem with their safety or efficacy are usually withdrawn for economic reasons.

List of Drug Products that Can Be Approved with Adequate Dissolution

After a page that contains a link to the Orphan Product Designations and Approvals list, there is a page officially called “Drug Products which must Demonstrate in vivo Bioavailability only if Product Fails to Achieve Adequate Dissolution.” These products have AB ratings in the Orange Book but bioequivalence trials are not required for them, as long as they meet FDA dissolution standards. The reason for this exception is that there is adequate scientific evidence that these products are very easily absorbed from the digestive tract. If the dissolution of the product is fast enough, it will be irrelevant to the therapeutic equivalence of the product.

There are probably many more products for which the previous sentence is true. However, there is not enough scientific evidence for FDA to be willing to add them to the list. In August 2000, FDA issued a Guidance, “Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System,” that was intended to remedy this situation. However, this approach has not been particularly successful as of the preparation of this chapter.

Appendices

Appendix A of the Orange Book is a list of brand names and the corresponding generic names of product in alphabetical order. Appendix B is an alphabetical list of NDA, BLA and ANDA applicants, with all their products listed alphabetically for each. It is the experience of this author that there are occasional errors in this list. Appendix C is a list of terms that FDA uses to describe dosage forms. FDA includes this list to encourage

uniformity in describing equivalent dosage forms. For example, FDA prefers Extended Release rather than sustained or controlled release for products designed to release their active ingredients over a long period of time.

Patent and Exclusivity Information

While not directly relevant to the subject of this volume, the Patent and Exclusivity Information section of the Orange Book is extremely important to pharmaceutical professionals. It is one of the few parts of the Orange Book that is not available anywhere else. Products are listed in this section alphabetically by their generic names, followed by their brand names. On the next line is the NDA number for the product. Next to this number is a list of patents that have been submitted to the FDA by the applicant in accordance with the rules of the 1984 amendments to the FDCCA. Each patent is accompanied by its expiration date. For certain patents there is also a “use code,” attempting to describe what uses of the product are covered under this patent. In some cases there are also codes indicating whether the patent is an drug substances (DS) or drug product (DP) patent.

In addition to the information described in the last paragraph, there is also information about any exclusivity the product may have been granted in the right two columns of this listing. Exclusivity was defined in the 1984 amendments to the FDCCA as a period of time granted to certain new or revised products during which FDA may not approve products that have the exclusive product as their reference listed drug. For more information on this process, the reader is referred to Chapter 5 of reference 2.

ELECTRONIC ORANGE BOOK AND DRUGS@FDA

For 15 to 20 years pharmaceutical professionals used the paper Orange Book, many of us so extensively that each year's copy threatened to fall apart before we received the next one. However, in the last several years the paper book has been largely supplanted by two excellent FDA web sites.

The older of the two sites is the Electronic Orange Book, which has been available since 1997. The home page link for this site is <http://www.fda.gov/cder/ob/default.htm>, and it is orange. From this home page the user can access several useful sets of information. The first choice is the Annual Edition, which provides pdf files of the Annual Edition and its Cumulative Supplements. These look just like the paper version. The second choice is FAQ, which takes the user to a choice of Frequently Asked Questions for the Orange Book and for Patent and Exclusivity information. This section is fairly new and still rather basic.

Following these choices there are five different ways to search the Electronic Orange Book. The user may search by active ingredient,

proprietary name, application holder, application number or patent. For the first four of these, the user must indicate whether the product is prescription (Rx), OTC or discontinued. This is also true for the first choice in search by patent, which is search by patent number. The second choice in search by patent allows the user to display all newly listed patents for the month, but the user must choose Rx, OTC or discontinued. The last choice returns a list of all patents that have been delisted from the current edition of the Orange Book.

Since about 2002, FDA has provided a second web site, called Drugs@FDA. The link to its home page is <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm>. This database is electronically updated from the Orange Book, but it contains valuable information not found in the Orange Book. The site contains a useful set of FAQ, an excellent glossary and several ways to search the data. The user just has to enter the first three or more letters of the drug name, either the brand name or the generic name, and the site will return a list of all approved products containing drugs that start with the letters entered. The user does not have to know whether the drug is OTC or generic, active or discontinued. The user does not even have to know how to spell the name, as long as the first letter is known, because the site provides a browsing list of all approved products that start with each letter of the alphabet.

The search will return a list of all products containing the drug name entered. Clicking on a brand name will give an overview listing for the brand product, including the drug name, application number, dosage form, route of administration, strengths, marketing status (prescription, OTC, discontinued or none if the product is only tentatively approved). If the user selects (clicks on) the name and application number a detailed list of each dosage form and strength will appear, indicating which is the reference listed drug. There is a link allowing the user to go to a list of all products that the FDA has declared to be therapeutically equivalent to the product initially selected.

There is also a link called "Approval History and Related Documents." The link takes the user to a list of actions FDA has taken on the application, starting with the approval or tentative approval. For very old products and/or discontinued products there may be no list, or just a few entries. For most other products the list will show all approved supplements, with the supplement number and a short description of what was approved, called the approval type. In many cases, the list also has links to FDA reviews, FDA approval letters, and/or approved labeling.

Before the FDA created the Drugs@FDA site, this information was very difficult to obtain. Responses to requests under the US Freedom of Information law took months or years. At times the FDA would respond that they could not find the information, perhaps because the request did not give quite enough details or was too broad. By posting this information,

FDA can communicate to everyone what processes and standards it used for approval of various products. For the purposes of this volume, the Clinical Pharmacology and Biopharmaceutics Reviews would be the most informative.

CONCLUSION

This chapter has discussed various types of information that is available from the FDA and how to find it. Such information can serve as a frame work for the discussion of various issues in the demonstration of bio-equivalence and therapeutic equivalence which occupy the remainder of this volume.

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Pharmaceutical Alternatives: Considerations for Generic Substitution

Roderick B. Walker

*Faculty of Pharmacy, Rhodes University,
Grahamstown, South Africa*

Roger K. Verbeeck

*School of Pharmacy, Université Catholique de Louvain,
Brussels, Belgium*

Isadore Kanfer

*Faculty of Pharmacy, Rhodes University,
Grahamstown, South Africa*

INTRODUCTION

The issue of interchangeability/switchability relating to pharmaceutical alternatives is a controversial one and poses a challenge to regulatory authorities in particular where the consideration of generic substitution is important (1). The term *pharmaceutical alternative* as defined in the EU guideline (2) is used to define pharmaceutical products that have the same active moiety but that may differ in chemical form (i.e., salt, ester etc.) of that active compound or in dosage form or strength. A similar definition exists in the text “Approved Drug Products with Therapeutic Equivalence Evaluations” (Orange Book) published by the Food and Drug Administration (FDA) (3). While both the European Agency for the Evaluation of Medicinal Products (EMA) and the FDA recognize the concept that pharmaceutical alternatives may be shown to be bioequivalent, the Orange Book (3) clearly states that only *therapeutic equivalents* that are *pharmaceutical equivalents* can be considered substitutable, whereas the EMA

states that either pharmaceutical equivalents or pharmaceutical alternatives may be considered as therapeutic equivalents provided that the excipients contained in the formulation do not impact on the safety and efficacy profile of the dosage form (2). Pharmaceutical equivalents are defined in the Orange Book (3) as drug products that contain the same active ingredient(s), are of the same dosage form, route of administration and are identical in strength or concentration amongst others. The EMEA further stipulates that in order to conclude that a product is therapeutically equivalent with another product, clinical evidence of the safety and efficacy of the test product must be forthcoming and therefore bioequivalence testing of two products containing different salts of the same moiety may not suffice to establish substitutability.

It is widely recognized that different formulations even though containing the same quantity of the same active drug can show significant differences in bioavailability, i.e., the rate ($C_{\max}$) and extent (AUC) of absorption of the active substance. Whereas FDA (4) for example requires that products must be pharmaceutically equivalent and bioequivalent to be prescribable and interchangeable the EMEA (2) uses a less conservative approach by allowing bioequivalent pharmaceutical alternatives that are not pharmaceutical equivalents to be considered therapeutically equivalent according to their definition and therefore by implication, substitutable.

The issue of pharmaceutical alternatives therefore and the establishment of therapeutic equivalence includes several considerations such as those related to different salts of the same moiety, differences in dosage forms and strengths and those relating to differences in routes of administration.

SALT FORMS OF DRUGS

It is well known that most drugs are either weak organic acids or bases and can therefore exist as different salt forms and that despite each active pharmaceutical ingredient (API) in a different salt being the same moiety, each of the salts may be considered as a distinct chemical entity with its own chemical and biological profile which may lead to differences in related clinical efficacy and safety profiles (5–8).

The conversion of an API to a particular salt form is a means of modifying and potentially optimizing specific and appropriate physico-chemical properties (8). However, a change in the salt form may also affect the biological properties of the drug and have significant implications for safety and toxicity (7). The most appropriate salt form of an API must ideally be selected at an early stage of the development of a new chemical entity, to optimize the characteristics of the final formulation. It has been estimated that half of all APIs used in medicinal therapy are administered as salts, and salt formation of potentially useful molecules has become an essential step in drug development (9,10).

Different salt forms of a particular API may differ substantially in their physicochemical properties, in particular solubility, hygroscopicity, stability, flowability, etc. In addition, the presence of impurities associated either with the route of synthesis of that particular salt or resulting as a consequence of instability and the formation of degradation products, can impart toxicity and/or undesirable biological activity quite different from the intended clinical use of the drug (11,12). Consequently, the use of one salt form of an API as opposed to another may result in a substantial difference in therapeutic efficacy with a resultant negative impact on the safety and/or quality of that specific molecule. There is no reliable way of predicting the influence of a particular salt species on the behavior of a parent compound in different dosage forms.

The selection of an appropriate form of an API is not only an important factor in the early stages of new drug product development (13) but is also a critical factor in the development of generic drug products. An interesting case is illustrated by the example of amlodipine. Amlodipine, a calcium channel blocker is marketed by Pfizer as a besylate salt and is commercially available as (Norvasc[®]). The original patent held by Pfizer on amlodipine besylate expired in 2003 but was extended until 2007 to compensate for a lengthy review process by the FDA (14). The original patent granted to the manufacturer protected both the chemical structure of amlodipine besylate and a series of other salts of amlodipine. A maleate salt product of amlodipine that was developed by Dr. Reddy's Laboratories Limited (AmVaz[™], Reddy Pharmaceuticals Inc.) was subsequently proved to be bioequivalent to Norvasc (15). Dr. Reddy's Laboratories claimed that Pfizer's patent extension did not apply to their version of the drug, i.e., amlodipine maleate. However, on February 27, 2004, The United States Court of Appeals for the Federal Circuit reversed an earlier New Jersey District Court's dismissal of Pfizer's patent infringement action against Dr. Reddy's Laboratories' generic version of Norvasc effectively preventing a generic version of amlodipine from entering the market (16).

Apart from the legal issues, an important question to be answered is: what experiments and tests are required to ensure that a drug product containing a specific salt form of an API has comparable pharmacokinetic, pharmacologic, toxicologic and safety profiles as the registered product containing an alternative salt form of the same active substance? Furthermore, what is the likelihood that pharmaceutical alternatives, which have been shown to be bioequivalent, will have different clinical safety and efficacy profiles?

As mentioned previously, different salt forms of an API may vary in their physicochemical characteristics including but not limited to solubility and hygroscopicity. Increased hygroscopicity may reduce the stability of an API even in a pharmaceutical dosage form such as tablets, in particular if the API is susceptible to hydrolytic degradation. Furthermore, thermal

stability and degradation pathways may differ for alternative salt forms of the same active moiety that may result in the need to evaluate new degradation products using appropriate toxicological and/or other studies.

Once again, amlodipine maleate provides an interesting example when compared to the besylate form of the molecule and where instability of the maleate salt results in the formation of a degradation product that has significant implications for safety and toxicity. The maleate salt of amlodipine, unlike the besylate salt has an intrinsic chemical instability which results in the formation of N-(2-{[4-(2-chlorophenyl)-3-(ethoxycarbonyl)-5-(methoxycarbonyl)-6-methyl-1,4-dihydro-2-pyridyl]methoxy} ethyl) aspartic acid. This impurity has demonstrated biological activity and is formed by an intramolecular reaction of the unsaturated maleic acid with the primary amine group of amlodipine. This aspartic acid derivative has been shown to possess a distinctly different biological profile to amlodipine itself. While low levels of the impurity may not result in serious clinical consequences, the instability of the maleate salt suggests that relatively high levels may be present following the manufacture of dosage forms and on prolonged storage.

Therapeutic equivalence between two medicinal products not only implies the same efficacy but also the same safety profile. The issues raised above related to the possible difference in toxicity and stability of the maleate and besylate salt forms of the amlodipine moiety, demonstrate that an alternative salt form may have to undergo toxicological evaluation, in addition to a valid study showing *in vivo* bioequivalence, before therapeutic equivalence, for example to a different (marketed) salt form of the same active moiety, can be accepted.

Additional issues that are also important when considering whether alternative salt forms of the same active moiety can be considered therapeutically equivalent and hence have to be addressed when developing a generic drug product using an alternative salt form of the active substance include: solubility, dissolution, bioavailability, toxicity, polymorphism, formulation and manufacturing considerations.

The dissolution rate of an active substance in the gastrointestinal fluids following liberation from its dosage form is primarily a function of the aqueous solubility of the API in question. Therefore, solid dosage forms containing alternative salts of the same active substance may show different *in vivo* dissolution characteristics. According to the principles underlying the Biopharmaceutics Classification System (BCS) for active drug substances with a high intestinal permeability, the *in vivo* dissolution rate will determine the rate and in some cases also the extent of absorption (17). Therefore, for an active substance with low intestinal permeability and relatively good aqueous solubility, the *in vivo* dissolution will not be the rate-limiting step in the absorption process and differences in aqueous solubility and dissolution are therefore not important determinants of bioavailability.

The scientific literature is replete with reports showing that the aqueous solubility of an API can be significantly modified by use of alternate salt forms of the same active moiety and that the solubilities of the different salt forms can be vastly different. The antidepressant, trazadone, for example, is currently marketed as the hydrochloride salt. In order to prepare a form of trazadone with lower aqueous solubility than the hydrochloride salt, a number of alternative salts have been prepared (18). Of the salts selected for evaluation, the tosylate and pamoate salts were found to be less water-soluble than the sulfate and hydrochloride salts and the most interesting solubility profile with values ranging from 3 mg/mL at pH 1.0 to 0.2 mg/mL at pH 12.0 was exhibited by the tosylate salt. The low aqueous solubility makes the tosylate salt the candidate of choice for the development of a prolonged release oral product for the elderly due to the potential for improved compliance in these patients. The significantly lower (8–10 fold in the pH range 1–5) solubility of the tosylate salt compared to the hydrochloride salt, may result in dissolution rate-limited absorption of trazadone following oral administration of the tosylate salt *in vivo*. The vast difference in solubility makes it highly unlikely that the two salts can be bioequivalent.

The impact of a difference in aqueous solubility of a specific salt on the therapeutic activity and duration of action of an API is further elucidated by evaluation of the solubility of the hydrochloride and napsylate salts of dextropropoxyphene. Dextropropoxyphene hydrochloride is highly soluble (1 in 0.3 parts water) whereas the napsylate salt is practically insoluble (1 in > 10,000 parts of water) (19). The more extensive analgesic activity and longer duration of action of the hydrochloride salt of dextropropoxyphene compared to the napsylate salt may in part be explained by the differences in solubility of the two salts (20). Furthermore the higher acute toxicity of dextropropoxyphene following administration of the hydrochloride salt compared to the napsylate salt following oral administration to mice is probably due to the faster absorption rate of the hydrochloride salt from the gastrointestinal tract (21).

Bioequivalence studies in humans in which different salt forms of basic drugs have been reported are rather limited and interestingly, none of them have reported significant differences in bioavailability between the different salt forms as a consequence of differences in their aqueous solubilities (22). For example, no enhancement in bioavailability was reported when salts of a basic antihypertensive agent with significantly different intrinsic dissolution rates were compared (23). Walmsley et al. reported no differences in the extent of bioavailability between the oxalate and citrate salts of naftidrofuryl (24) and Jamuludin et al. reported no significant differences in $C_{\max}$, $T_{\max}$, or AUC of quinine following oral administration of the hydrochloride, sulfate, and ethyl carbonate salts to healthy volunteers (25).

Clearly, an *in vivo* bioequivalence study is necessary if therapeutic equivalence between alternative salts of the same active drug molecule is to be claimed, except when both salts are highly soluble and highly permeable, i.e., BCS class I compounds. In that case a BCS based waiver for an *in vivo* BE study for an immediate release oral dosage form which exhibits rapid *in vitro* dissolution can be requested, provided a number of additional conditions are met (26).

The toxicity of a salt of an API may be due to the conjugate anion or cation used to form the salt (5,8). Pravastatin maleate exhibits nephrotoxicity and has been reported to cause renal tubular lesions in the dog as a result of the formation of maleic acid from the maleate anion used to form the salt (27).

The safety profile of a salt-forming agent depends largely on its chemical nature and its biological characteristics and the need to investigate the toxicity of a specific salt forming agent will depend, to a certain extent, on whether the agent has previously been used in other medicinal products, foods and beverages as well as the relative ratio of the salt-forming component to active substance.

Toxicity studies are required for all new salt forms of an active substance when the salt of that active substance has been prepared from a new salt-forming agent with little or no information on its toxicity profile. Toxicity studies on the salt-forming agent alone are also necessary. Of particular importance to the developers of new drug products is that the monographs on 68 salt-forming acids and 27 salt-forming bases have been published in the *Handbook of Pharmaceutical Salts: Properties, Selection and Use*, edited by Stahl and Wermuth (8) as well as a comprehensive list of salt forming acids and bases with information regarding their safety/toxicity (28,29).

The differences found in toxicity profiles of various salt forms of an API may be due to potentially toxic chemical impurities formed during the preparation of a specific salt of an API. It is therefore essential to evaluate the toxic potential of all impurities formed and isolated during the synthesis of a specific salt form of an API (30). By way of example, methane sulfonic acid is used for the preparation of methane sulfonates or mesylate salts of active basic drug molecules such as pergolide, nelfinavir, imatinib and amlodipine. In addition, benzene and toluene sulfonates or besylate and tosylate salts respectively have also been prepared. The potential health hazards of trace amounts of mesylate esters, including methyl methanesulfonate, ethyl methanesulfonate and isopropyl methanesulfonate, in pharmaceuticals have attracted the attention of health authorities (31). The impurities are formed by the reaction of methane sulfonic acid with solvents such as methanol, ethanol and isopropyl alcohol during the manufacture of mesylate salts of active substances. Furthermore, the use of alcoholic solvents during the manufacture of dosage forms may also precipitate the

formation of these impurities resulting in the production of potentially unsafe dosage forms. Mesylate esters are known to be potent mutagenic, carcinogenic and teratogenic compounds (32,33). Therefore it can be concluded that when routes of synthesis to manufacture and prepare different salt forms of the same API result in different chemical by-products, the toxic potential of these impurities should be evaluated by preclinical testing for each salt form synthesized/prepared.

In addition to issues of safety and toxicity, the tolerability of an API may also be affected by the specific salt form of an active substance administered by specific routes of administration. The potential of an API to cause gastrointestinal irritation and/or ulceration, for example, may in part be dependent on the aqueous solubility and dissolution rate of different salt forms of that API administered via the oral route.

For example the ulcerogenic effects of five different salts of alprenolol were compared to a placebo in a porcine esophageal test model (34). The highly water soluble hydrochloride and fumarate salts of alprenolol gave rise to the highest plasma concentrations of API yet evoked serious oesophageal lesions, while the salts with low solubility, the benzoate, maleate and sebacate salts produced no irritant effects on the esophagus. Furthermore the plasma levels of alprenolol were much higher after administration of alprenolol hydrochloride in the esophagus than after an identical intraduodenal dose of the same salt possibly due the avoidance of hepatic first-pass metabolism/degradation following oesophageal absorption.

The solid-state properties of a molecule, as well as its properties in solution, can be modified by use of salt formation. The selection of a salt suitable for a specific route of administration or a particular dosage form of a drug substance requires that all relevant solid-state properties of a salt candidate be thoroughly investigated prior to the continuation of product development.

Polymorphism is frequently a critical point in determining the preference for one salt or another (9,13). Polymorphism is defined as the ability of a drug substance to exist as two or more crystalline phases that have distinct molecular structures and/or conformations of the molecules in the crystal lattice. Polymorphism is a widespread phenomenon observed in over 60% of all API's and the most critical issue related to polymorphism of an API is the equilibrium solubility which is an important determinant of dissolution rate and which in turn may affect the bioavailability of the active substance particularly following oral administration (35).

There are numerous examples where polymorphism has been associated with differences in the oral bioavailability of an active substance from solid dosage forms, including chloramphenicol palmitate and carbamazepine base (36,37). Consequently it is essential that the production of different salts in order to overcome solubility and other challenges must necessarily involve an investigation into the formation of polymorphic

forms, such that the correct form of the API is produced for the purposes of large scale manufacture of an API and subsequent dosage form production.

DIFFERENT DOSAGE FORMS

The key considerations in this discussion revolve around the issue of pharmaceutical alternatives being considered therapeutically equivalent and therefore interchangeable following an appropriately designed study to prove bioequivalence. In other words, if pharmaceutical alternative dosage forms are found to be bioequivalent, what precludes them being considered interchangeable? The answer to this question is moot since the FDA (4) does not permit substitution if a product is deemed to be bioequivalent but not a pharmaceutical equivalent whereas the EMEA (2) guideline provides for a pharmaceutical equivalent or pharmaceutical alternative to be considered therapeutically equivalent if the products are bioequivalent. A further complication involves the consideration of different routes of administration where clearly two products with different routes of administration cannot be pharmaceutical equivalents. In the EMEA Note for Guidance (2) the statement relating to pharmaceutical alternatives is silent regarding the route of administration. This could be interpreted that pharmaceutical alternatives intended for administration by different routes and that have been shown to be bioequivalent could be considered to be therapeutically equivalent.

In the case of a BCS Class I (17) compound that meets the necessary requirements for a biowaiver as defined (4), a product need not undergo a bioequivalence assessment. Therefore an API delivered from a tablet compared with a capsule qualifies for a biowaiver and the need for the product to be pharmaceutical equivalent is indeed questionable with respect to substitution. Is this not therefore a case for a waiver of the FDA's pharmaceutical equivalent requirement?

An example of different dosage forms being found to be bioequivalent has been shown for loperamide following administration of film coated tablets and capsules to 24 male volunteers in a randomized two-way crossover study using the conventional AUC acceptance criterion of 0.80 to 1.25 and an extended confidence interval (CI) of 0.75 to 1.33 for $C_{\max}$ that had been stated a priori (38). In this instance these pharmaceutical alternatives may be considered substitutable according to the EMEA (2) guidelines.

Another example of pharmaceutical alternatives that were found to be bioequivalent has been reported for lansoprazole in a study in which 15 and 30 g sachets for suspension in water were found to be bioequivalent to intact capsules when compared in a study in which 36 subjects were dosed in a crossover design (39).

Nasogastric tubes for the delivery of crushed tablets usually intended for oral administration are often used in hospitals and home care environments. In order to determine whether this alternate route of administration and essentially administration of a pharmaceutical alternate dosage form would produce equivalent responses, the antibiotic, trovafloxacin, was administered to 24 healthy volunteers in a four-period, four-treatment crossover study (40). The primary purpose of the study was to assess whether the use of an enteral feeding solution and location of the nasogastric tubes affected bioavailability of the antibiotic. The subjects were administered either two 100 mg tablets orally, two crushed tablets (pharmaceutical alternative) suspended in water via a nasogastric tube into the stomach, two crushed tablets suspended in water into the duodenum or two crushed tablets suspended in water and administered simultaneously with an enteral feeding solution into the stomach. The study in fact deals with two issues that are related to differences in route of administration and the administration of a pharmaceutical alternative dosage form in the form of crushed tablets. The results indicated that the treatment in which crushed trovafloxacin tablets were administered into the duodenum revealed bioinequivalence whereas delivery of the same pharmaceutical alternative via nasogastric tube into the stomach proved to be bioequivalent to the orally administered tablets (40).

A further illustration of bioequivalence between two different dosage forms has been recorded following the administration of 60 mg of citalopram to 24 subjects of mixed sex, the 90% CI for AUC_{last} and C_{max} were found to fall within the conventional limits for bioequivalence indicating that the two formulations can be considered bioequivalent (41).

Plasma levels of nizatidine administered to 24 healthy adult subjects and delivered from a commercial oral syrup formulation and two extemporaneously prepared liquid formulations in apple juice and infant formula were compared to those obtained following administration of a nizatidine capsule (42). The results indicated that the commercial oral syrup and extemporaneous infant formula liquid dosage form (pharmaceutical alternatives) were bioequivalent to the reference capsule using a conventional 90% CI for AUC_{last} and C_{max} whereas a possible food effect was observed for the extemporaneous apple juice formulation resulting in bioinequivalence in that comparison.

Plasma levels of levetiracetam were compared following administration of a 10% levetiracetam (750 mg) oral solution and 750 mg tablets in a crossover study in 24 healthy subjects and these two pharmaceutical alternative products were found to be bioequivalent (43).

A new rapidly disintegrating cisapride (Propulsid Quicksolv) formulation was compared to conventional cisapride (Propulsid) tablets in 36 elderly volunteers in a crossover study and found to be bioequivalent with both AUC_{last} and C_{max} ratios falling within the established limits for

bioequivalence (44) at a 90% CI. Similarly, in a crossover study in 40 healthy volunteers of mixed sex, an orally disintegrating dosage form of mirtazapine was found to be bioequivalent to a conventional tablet and the 90% CI fell into the generally accepted range of 0.8 to 1.25 for all parameters (45). Both these examples allude to pharmaceutical alternatives that could be acceptable for generic substitution.

It is evident that there are certain instances in which pharmaceutical alternate dosage forms are bioequivalent and in such cases may be interchangeable. However, substitution of these alternate dosage forms should be made with circumspection since there are biopharmaceutical and physiological considerations that should be evaluated prior to the substitution.

CONTROLLED RELEASE DOSAGE FORMS AND MECHANISM OF RELEASE

The Orange Book (3) considers different dosage forms and strengths within a product line by a single manufacturer to be pharmaceutical alternatives. It then goes on to infer that an extended-release product when compared with immediate-release or standard-release formulations of the same active ingredient is also a pharmaceutical alternative. However consideration has been given to the specific release mechanisms whereby extended release products with different rate controlling elements require to be tested against a reference listed drug having the same mechanism of release (3). For example, a test product containing nifedipine and formulated with a release mechanism in which osmotic pressure is the driving force for release will need to be compared with Procardia® XL, Pfizer, whereas a formulation in which the release of active substance is not based on osmotic principles must be compared with Adalat® CC, Bayer Pharma, to qualify for an AB rating. This proviso suggests that extended release dosage forms with different release mechanisms are not considered to be pharmaceutical equivalent and by implication are therefore different dosage forms differentiated only by mechanism of release. In the nifedipine example both products are tablets but are implied to be pharmaceutical alternatives and would not be substitutable even if bioequivalence was established. The EMEA (2) on the other hand may permit substitution of such products if proven bioequivalent.

Pharmaceutical alternatives containing sodium valproate as modified release granules were compared with two different sustained release tablet formulations (46). The modified release granules were found to be bioequivalent to both sustained release formulations in both the fed and fasted state.

The steady state bioavailability of two novel delivery systems containing metoprolol succinate or fumarate in a multiparticulate and an oral osmotic system (OROS®) respectively was investigated (47). The dosage forms were both of the reservoir type in which a drug core was surrounded by a release-controlling membrane but they function on different

formulation principles in which succinate salt containing beads were included into a disintegrating tablet whereas the fumarate salt was included in the nondisintegrating OROS[®] system. The products were found to be bioequivalent using a 90% CI of 0.8 to 1.25 for both $C_{\max}$ and AUC although the variability associated with the multiple-unit system was lower than with the single-unit device (47).

The example described above demonstrates a pharmaceutical alternative, which both involves different salts in addition to different dosage forms and yet was shown to be bioequivalent.

DIFFERENT ROUTES OF ADMINISTRATION

It is well known that the route of administration and type of delivery system may impact on bioavailability and hence pharmaceutical alternatives intended for a different route of administration compared to the reference product are quite unlikely to be shown to be bioequivalent.

An interesting case involves the bioavailability and bioequivalence of the same dose of etodolac administered as either a tablet or a suppository formulation in healthy volunteers of both sexes. In a crossover design when these different dosage forms were compared, their AUC_{last} values were found to be within the bioequivalence acceptance range for that parameter but not their $C_{\max}$ values (48), hence bioequivalence cannot be claimed.

A further example that demonstrates the importance of considering differences between routes of administration for the same product can be gleaned from the following. When hydroxypropyl methylcellulose or gelatine capsules containing ibuprofen, i.e., two pharmaceutical alternatives intended for oral administration, were administered rectally, there were significant differences in bioavailability between these formulations indicating bioinequivalence. However when the same capsules were administered orally in a crossover study the products were found to be bioequivalent (49). The two capsule formulations are by definition not pharmaceutical alternatives but rather pharmaceutical equivalents and would be substitutable when administered orally but not rectally. Clearly in this case the impact of excipients on drug release with specific routes of administration is evident.

Consequently the use of bioequivalent pharmaceutically equivalent products should only be considered substitutable provided the same route of administration is used.

The definition of a pharmaceutical equivalent clearly states that such dosage forms must be formulated for delivery via the same route of administration as well as the other considerations including amount and type of active moiety. However the definition of pharmaceutical alternative dosage forms makes no reference to the route of administration and also provides for different strengths, different salts of active moieties and different dosage forms.

Hence it would be prudent to consider limiting substitution of bioequivalent pharmaceutical alternatives to pharmaceutical alternatives that are formulated in different dosage forms but administered by the same route.

Although studies showing bioequivalence using pharmaceutical alternatives administered by different routes of administration are conspicuous by their absence in the literature, several such studies have been reported where the products were shown to be bioinequivalent for example, etodolac following oral and rectal administration (48) and zolmitriptan following nasal and oral administration (50). It may therefore be possible to show bioequivalence between dosage forms of different strength delivered by different routes of administration. Could a case be made for generic substitution of such products?

CONCLUSIONS

The issue of pharmaceutical alternatives for use in generic substitution is clearly quite controversial. While the EMEA Note for Guidance (2) indicates that pharmaceutical alternatives can be therapeutically equivalent under certain conditions some member states allow substitution whereas other member states do not. The latter is the same as the FDA's ruling. However when pharmaceutical alternatives involve the use of different salts of API's it is critical that the safety and toxicity studies of such alternative dosage forms must be confirmed prior to permitting the use of these pharmaceutical alternatives as substitutes.

The substitution of different dosage forms and/or strengths of such dosage forms, administered by the same route after being proven bioequivalent in an appropriately designed well controlled study may be acceptable (2). The outcomes of substituting different strengths of different dosage forms delivered by alternate routes which have been shown to be bioequivalent are, however, currently unknown. Hence the implications of generic substitution of such pharmaceutical alternatives need further investigation.

In the case of controlled/modified/extended release or novel delivery systems that involve different release mechanisms the factors that mitigate against generic substitution of such products which have been shown to be bioequivalent are not readily apparent.

In conclusion, it is clearly evident that all data be carefully considered in permitting the use of pharmaceutical alternatives for generic substitution.

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Pharmacodynamic Measurements for Determination of Bioequivalence

Manish Issar

Watson Laboratories, Inc., Corona, California U.S.A.

Jeffrey G. Stark

Cedra Corporation, Austin, Texas U.S.A.

Leon Shargel

*Applied Biopharmaceutics,
Raleigh, North Carolina, U.S.A.*

Bioavailability is defined by the U.S. Food and Drug Administration (FDA) as “*the rate and extent to which the active ingredient or the active moiety is absorbed from a drug product and becomes available at the site of action.*” Whereas the measurement of drug concentrations in biological fluids provides information on the bioavailability of drugs intended to be absorbed into the systemic circulation, for drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action” (1,8). The latter approach may be accomplished by using pharmacodynamic endpoints in certain cases where the direct chemical measurement of the active drug substance in biological fluids is not feasible.

PHARMACODYNAMICS

Pharmacodynamics refers to the relationship between the drug concentration at the site of action (receptor) and the pharmacologic response. The pharmacologic response that is produced depends upon the chemical structure of

the drug substance and its interaction with the drug receptors at the site of action. The nature of most pharmacologic actions is assumed to be reversible and conforms to the *Law of Mass Action*. The quantitative relationship describing a single drug molecule interacting with a receptor site to produce a pharmacologic response is given below:



The brackets denote molar concentrations. In this relationship, the occupancy of the drug molecule at the receptor site does not change the affinity or the ability of more drug molecules to complex with the receptor at additional drug sites. Other pharmacodynamic models have also been described (2,3).

The onset, intensity and duration of the pharmacodynamic response depend upon the dose and the pharmacokinetics of the drug. As shown in Equation (1) above, as more drug is absorbed into the body, more drug reaches the receptor site and the pharmacodynamic effect increases to a maximum effect. A linear graph relating the pharmacodynamic effect to drug dose shows a hyperbolic relationship (Fig. 1). If the same data are plotted on a log scale, a sigmoidal curve results (Fig. 2). Pharmacodynamic measurements have been used for many years to show differences in bio-availability. The relationship of the drug concentration versus time curve and the pharmacodynamic effect versus time curve are shown in Figures 3 and 4. The pharmacodynamic response is observed when the active drug substance is delivered to the site of action in an effective concentration. For example,

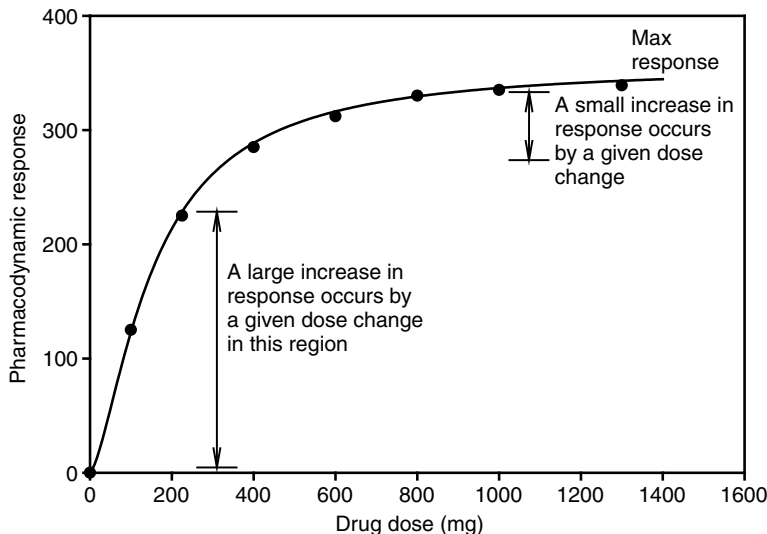


Figure 1 Pharmacodynamic response versus dose on a linear scale.

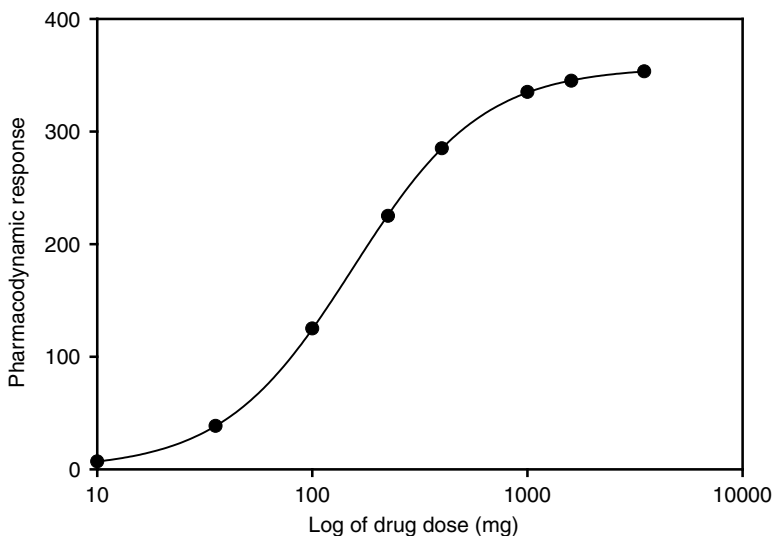


Figure 2 Pharmacodynamic response versus dose on a log scale.

the effect of an antibiotic has been observed by the inhibition of microbiological growth. The drug concentration that just inhibits the growth of bacteria is known as the *minimum inhibitory concentration* or MIC. This drug concentration could also be considered as the *minimum effective*

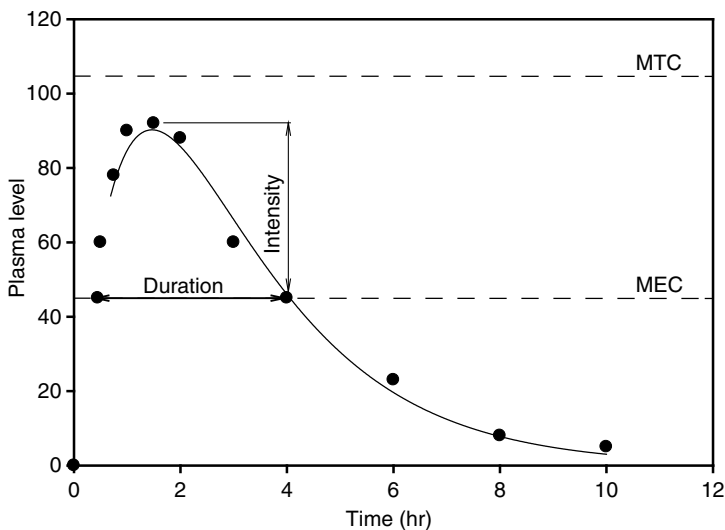


Figure 3 Plasma concentration versus time curve after oral administration of a drug. *Abbreviations:* MEC, minimum effective concentration; MTC, maximum toxic concentration.

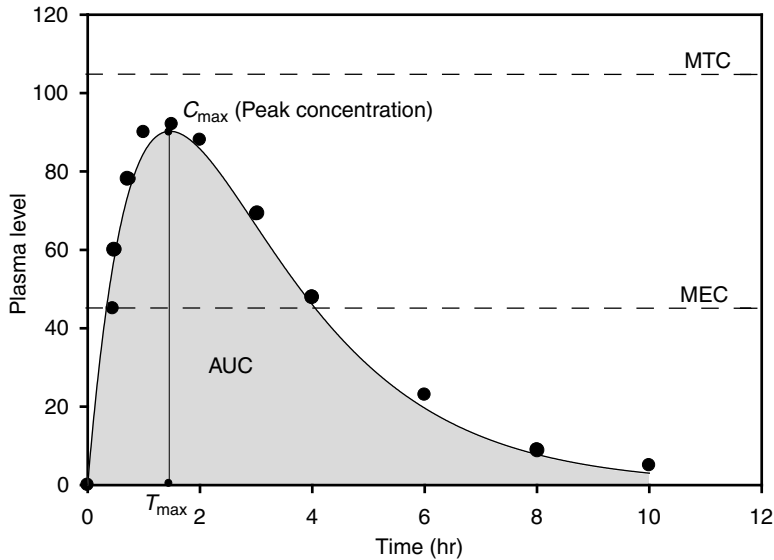


Figure 4 Plasma concentration versus time curve after oral administration of a drug. The shaded portion represents the area under the curve (AUC).

concentration (MEC). The *onset time* corresponds to the time required for the drug concentrations to reach the MEC or minimum drug concentration that just produces a pharmacodynamic effect. As long as the drug concentration is above the MEC, the drug effect continues. The pharmacodynamic response increases with an increase in drug concentration at the receptor site up to a maximum pharmacodynamic response. Thus, the maximum pharmacodynamic response is related to the *intensity* of the drug response as shown in Figure 3. Due to drug elimination, the drug concentration will fall below the MEC. The length of time that the drug remains above the MEC is known as the *duration* of drug activity. The time course for the plasma drug concentration curve may not have the same shape as the effect versus time curve. The time course for the drug to distribute in and out of the site of action and the maximum pharmacodynamic effect may occur before or after the maximum drug concentration is observed in the plasma.

EXPOSURE RESPONSE RELATIONSHIP

During new drug development, the relationship between drug dose to drug concentrations and the corresponding pharmacodynamic response is critical for determination of safety and efficacy (4–6). The U.S. FDA uses the term “*exposure*” to refer to the dose (drug input to the body) and various measures of acute or integrated drug concentrations in plasma and other biological fluid (e.g., $C_{\max}$ and AUC). *Response* refers to a direct measure of the pharmacologic

effect of the drug. Response includes a broad range of endpoints or biomarkers ranging from the clinically remote biomarkers (e.g., receptor occupancy) to a presumed mechanistic effect (e.g., ACE inhibition), to a potential or accepted surrogate (e.g., effects on blood pressure, lipids, or cardiac output), and to the full range of short-term or long-term clinical effects related to either efficacy or safety. Exposure-response data can also be used to demonstrate differences in safety and efficacy of a new formulation or a “to be marketed” formulation that differs somewhat from the formulation used in clinical trials. In this case, a difference in systemic drug exposure that is not clinically relevant may be observed between the two formulations if the bioequivalence (BE) measures, C_{max} and AUC, fall outside the 90% confidence intervals (CI) of 80% to 125%.

Biomarkers, Surrogate and Clinical Endpoints

A biological marker or *biomarker* refers to a biological response that can be used for a confirmation of diagnosis, monitoring drug treatment, monitoring disease progression, prediction of clinical outcome and assessment of systemic drug exposure (7).

Examples of biomarkers (Table 1) may include, in vitro cell based bioassays such as receptor binding assays, in vivo assays that measure neutrophil count to assess the effect of granulocyte-colony stimulating factor and early viral load reduction in chronic hepatitis C to assess the effect of alpha interferons. A *surrogate endpoint* is considered a subset of a biomarker that is intended to serve as a substitute for a clinically relevant

Table 1 Examples of Biomarkers and Surrogate Endpoints

Drug	Therapeutic indication	Biomarker/surrogate endpoint
Iron sucrose injection	Anemia	Iron stores and new hemoglobin synthesis
Alendronate sodium	Osteoporosis	Bone mineral density
Timolol maleate	Glaucoma	Intra-ocular pressure
Somatropin	Human growth hormone	Insulin-like growth factor-1
Insulin	Diabetes	Serum glucose
Sargramostim (granulocyte-colony stimulating factor)	Stimulate the production of white blood cells, especially granulocytes and macrophages, following chemotherapy	Absolute neutrophil count
Alpha interferon	Hepatitis C	Early viral load reduction in chronic hepatitis C

endpoint. The choice of biomarker and its relevancy should be justified and the method properly validated.

The measurement of plasma drug concentrations for bioavailability and BE studies can be considered as a surrogate marker for in vivo drug product performance. The use of plasma drug concentrations as a measurement of BE is one part of the requirements for demonstrating therapeutic equivalence to a reference listed drug product. The FDA “believes that products classified as therapeutic equivalent can be substituted with the full expectation that the substituted product will produce the same clinical effect and safety profile as the prescribed product” (8). Thus, a generic drug product is generally approved without performing a clinical outcome study. Additionally, plasma drug concentrations are often used as a direct measurement of exposure, which is generally related to drug efficacy and safety.

Drug Dose Response Curve

The relationship between the drug dose and the pharmacodynamic response is shown in Figure 1 (linear response) and Figure 2 (log dose response). On a linear scale, the relationship between the drug dose and the pharmacodynamic response results in a hyperbolic curve (Fig. 1); whereas, on a log scale, the relationship between the drug dose and the pharmacodynamic response results in a sigmoid curve (Fig. 2). Since the drug dose (mg) is distributed in a constant volume (mL) known as the volume of distribution, the same pharmacodynamic relationship would be observed when the drug

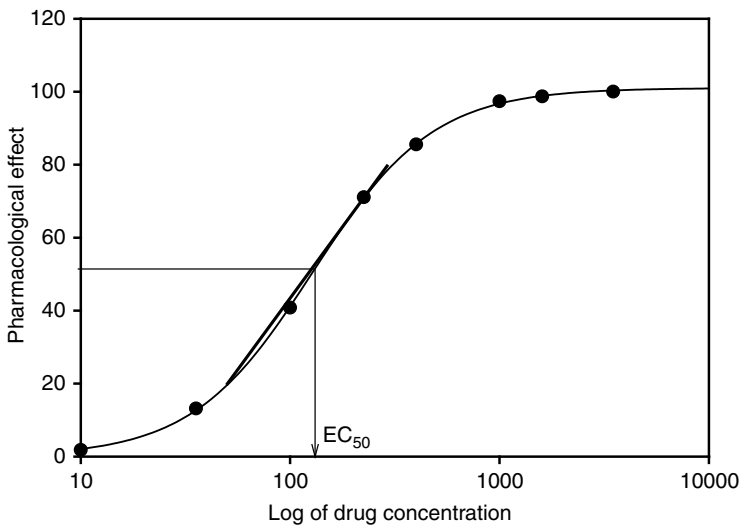


Figure 5 Log dose versus pharmacologic response curve. Bold line represents the linear portion (20 to 80%) of the curve.

concentration is plotted against time (Fig. 5). In practice, drug concentrations between 20% and 80% maximum response will give a linear response when using log drug concentrations.

The drug concentration that gives 50% of the maximum pharmacodynamic effect is known as the EC_{50} (Fig. 5). When relating drug concentrations to a pharmacodynamic effect, particularly for bioavailability and/or BE studies, drug doses should produce drug concentrations that are close to the EC_{50} . This portion of the drug concentration versus pharmacodynamic effect curve is most sensitive to changes in drug bioavailability from a drug product. BE studies of test and reference drug products that use pharmacodynamic endpoints must be able to distinguish whether there are any significant differences in the drug bioavailability between the two products.

Maximum Effect ($E_{\max}$) Model

The relationship shown in Figure 1 may be expressed mathematically by the following equation:

$$E = \frac{E_{\max}C}{EC_{50} + C}, \quad (2)$$

where E is the pharmacodynamic response at drug concentration, C . $E_{\max}$ is the maximum pharmacodynamic effect and EC_{50} is the drug concentration that produces 50% maximum pharmacodynamic effect. Equation (2) is an empirical model that is based on a saturable process similar to *Michaelis-Menton* enzyme kinetics and Equation (1). Figure 1 shows that it is difficult to observe differences in drug dose or drug bioavailability if the resulting drug effect is close to the $E_{\max}$.

PERFORMANCE OF PHARMACODYNAMIC STUDIES

When a pharmacodynamic effect study is used as a measurement of bioavailability and/or BE, a preliminary study may be needed to demonstrate a dose-response curve. Ideally, the dose of drug to be used should be close to the ED_{50} so that small changes in the fraction of drug absorbed from the drug product are observed by a proportional change in the pharmacodynamic response. For drug products whose doses are close to the $E_{\max}$, a difference between drug products is difficult to observe (even if such differences exist).

In some cases, subjects need to be pre-screened for responders and non-responders. This should be done prior to the study and the procedure should be stated in the protocol. The non-responders should be identified and excluded from the study. The methodology should be validated for precision, accuracy, reproducibility and specificity.

The pharmacodynamic response should be measured quantitatively using a double blind approach. If possible, measurements should be recorded by suitable instrumentation. If not available, a visual analogue scale may be used. If a placebo effect is expected, then the study design should consider a third (placebo) treatment group (see Chapter 5).

Pharmacodynamic Examples

Some examples of drugs with measurable pharmacodynamic endpoints that might be used in a BE study are listed in Table 2. Before executing a BE study based on pharmacodynamic endpoints, care must be taken to show that the dose–effect and time–effect curves are able to discriminate drug bioavailability between two drug products. Thus, the dose–response curve and the time of observation should be carefully evaluated. In addition, subjects that respond to the drug should be selected to maximized uniformity in response.

The dose used in the BE study should be selected so that the pharmacodynamic response obtained is within the linear phase, 20% to 80% of the dose response curve, and is able to discriminate bioavailability differences between two formulations (Fig. 5).

TICLOPIDINE HYDROCHLORIDE TABLETS

For example, ticlopidine hydrochloride (Ticlid[®]), is an antiplatelet drug that shows no antiplatelet activity per se but must be converted in vivo to its active metabolite. Studies have shown that pharmacokinetic parameters like $C_{\max}$ of ticlopidine (2–3 hours) do not correlate well to its maximum effect ($E_{\max}$) as there is a delayed response in the antiplatelet activity. Similarly the platelet recovery (11–13 days) obtained after withdrawal of ticlopidine seems to be much longer than the elimination half-life (1.4–4.1 days) for ticlopidine (9). These investigators tried to demonstrate BE of two ticlopidine preparations by ex vivo measurement of platelet aggregation induced by adenosine diphosphate (ADP). Platelet inhibition is the desired therapeutic outcome for ticlopidine. The antiplatelet activity is represented by the active metabolite hence measurement of the metabolite could be a pharmacokinetic way to demonstrate BE. The lack of information pertaining to quantitative aspects of ticlopidine metabolism justifies pharmacodynamic measures to show BE. Therefore measurement of platelet inhibition activity rather than traditional comparison of blood levels of the pro-drug (ticlopidine) could be considered a better predictor of biological activity and thus a better surrogate marker to demonstrate BE of two ticlopidine products. Figure 6 shows the mean ($\pm$ SD) plot of % free platelets with time obtained after ex vivo addition of 30 μ M of ADP to whole blood. The 90% CI for Max_{inh} and $\text{AUC}_{\text{inh}1-16}$ ratios were within 80% to 125%

Table 2 Examples of Drugs with Pharmacodynamic Endpoints

Drug	Route of administration	Drug class	Pharmacodynamic endpoint	Reference(s)	Comments
Procaterol hydrochloride 20 µg	Inhalation	β ₂ -adrenergic receptor agonist	FEV ₁	21	BE study between Meptin (HFA) and Meptin (CFC) metered dose inhalers (MDI). 90% CI for differences between the two treatments in mean AUC (FEV ₁)/h and mean peak FEV ₁ were within acceptance criteria of –0.15 to 0.15 (L)
Albuterol	Inhalation	β ₂ -agonist (bronchodilator)	PD ₂₀ FEV ₁	17	Methacholine challenge
Clobetasol 17-propionate (0.05% w/w)	Transdermal/topical	Topical corticosteroid	Skin blanching (vasoconstrictor assay)	22	BE studies for dermatologic products need to show a pilot dose duration-response and pivotal BE studies using reflectance colorimetry
Pindolol	Oral	β-blocker	Blood pressure	23	
Recombinant granulocyte colony-stimulating factor (G-CSF)	Subcutaneous	Peptide	Absolute neutrophils (ANC), white blood cells (WBC) and CD34+ cells count	24	

(Continued)

Table 2 Examples of Drugs with Pharmacodynamic Endpoints (*Continued*)

Drug	Route of administration	Drug class	Pharmacodynamic endpoint	Reference(s)	Comments
Ticlopidine 250 mg tablets	Oral	Antiplatelet	Inhibition of platelet aggregation	25	ACE inhibition is not a very sensitive marker for assessing BE as it would be difficult to find differences in the two products
Enoxaparin	Intravenous and subcutaneous	Anticoagulant	Anti-activated factor X (anti-FXa)	26	
Certoparin		Anticoagulant	Anti-activated factor X (anti-FXa)	26	
Glyceryl trinitrate	Sublingual spray	Anti-anginal	DPG changes (c/a: ratio of c-incisure and systolic a-wave)	27,28	
Fomoterol fumarate	Inhalation	Selective β_2 -agonist (bronchodilator)	PD ₂₀ FEV ₁	12	
Enalapril maleate	Oral	Antihypertensive	Serum ACE activity	29	
Recombinant interferon (IFN) α -2b	Subcutaneous	Antiviral, antiproliferative, immunomodulatory protein	Body temperature increase and β 2-microglobulin	30	

Abbreviation: BE, bioequivalence.

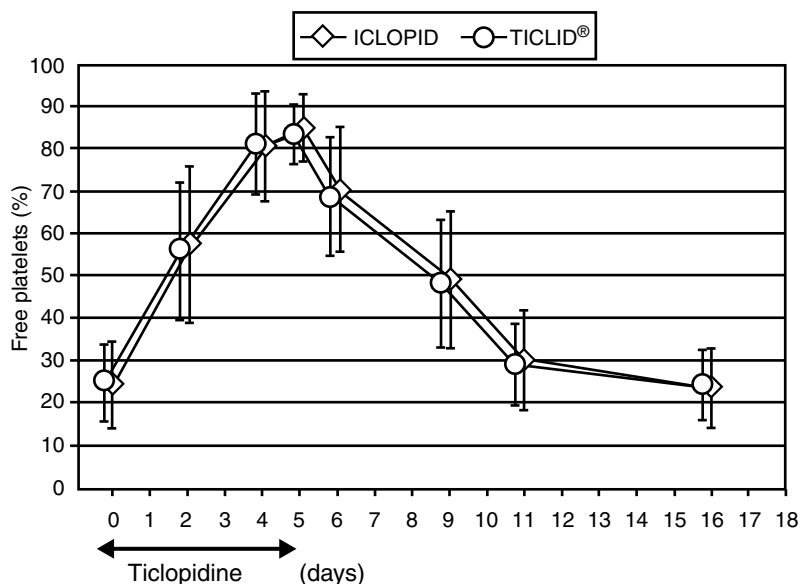


Figure 6 Ticlopidine BE study. Means ($\pm$ SD) of free platelets remaining in whole blood after addition of ADP ($30\mu\text{M}$) ex vivo in subjects who had received ticlopidine (Iclopil or Ticlid) 250 mg daily from day 0 (after blood sampling) to day 4 (arrow). Response to ADP included recovery period and was followed by day 16 (8). *Abbreviation:* ADP, adenosine diphosphate.

intervals (Table 3). Since a bioanalytical method is available for ticlopidine, a BE study for a generic ticlopidine hydrochloride tablet would be based on plasma drug concentrations rather than a pharmacodynamic endpoint.

Table 3 Ticlopidine Bioequivalence Study

Parameter	Geometric mean		Point estimate (T/R)	90% CI	
	Iclopil (test)	Ticlid (reference)			
$\text{AUC}_{\text{inh } 1-16}$	755.3	749.8	1.0075	0.9725	1.0438
Max_{inh}	86.2	85.3	1.0090	0.9907	1.0276
$T_{\text{max inh}}$	5.66	5.58	1.0148	0.9878	1.0426
$T_{05 \text{ inh}}$	6.34	6.34	0.9731	0.8818	1.0739

Abbreviations: $\text{AUC}_{\text{inh } 1-16}$, area under the curve platelet inhibition from day 1 to 16; Max_{inh} , maximal inhibition of platelet aggregation; $T_{\text{max inh}}$, time (days) at which maximal inhibition of aggregation occurred; $T_{05 \text{ inh}}$, half time in days of recovery of platelet activity based on the slope of the curve of platelet recovery following drug withdrawal.

Source: Data adapted from Ref. 9.

ENOXAPARIN SODIUM INJECTION

Enoxaparin sodium injection (Lovenox[®]) is a sterile aqueous solution containing a low molecular weight heparin that has antithrombotic properties. A direct chemical assay for enoxaparin sodium injection in plasma or serum is not available. The pharmacokinetics of enoxaparin sodium is determined by the use of bioassays based on specific anticoagulant activities, such as Anti-Xa and Anti-IIa (10,11). Based on antithrombotic properties (anti-Xa), the pharmacokinetics of enoxaparin sodium is linear after subcutaneous administration, in doses from 20 to 80 mg using anti-Xa AUC measurement (Table 4). The bioavailability of enoxaparin sodium after multiple doses has also been reported by the manufacturer (Table 5). These data show that the use of pharmacodynamic endpoints such as anti-XA and anti-IIa can be used to describe the pharmacokinetics of enoxaparin sodium. As shown in Table 5, the bioavailability of enoxaparin sodium can be described in terms of A_{max} (maximum drug activity) and AUC (area under the drug activity vs. time curve). Thus, pharmacodynamic endpoints for enoxaparin sodium can be used to determine the BE of various formulations of enoxaparin. However, to meet the criteria for therapeutic equivalence, the pharmaceutical equivalence of various enoxaparin sodium formulations would also have to be demonstrated.

FORMOTEROL FUMARATE INHALATION POWDER

Formoterol fumarate (Foradil[®]) is a long acting β_2 -selective adrenoreceptor agonist that is therapeutically used as a bronchodilator in patients suffering from coronary obstructive pulmonary disease. A very low dose of 12 μ g formoterol by the inhalation route poses the biggest challenge to quantify the parent drug in the systemic circulation. Apart from the analytical challenges, the FDA requires that a pharmacodynamic BE study be

Table 4 Dose Escalation Study for Enoxaparin Sodium

Parameter	Units	Enoxaparin dose			
		20 mg	40 mg	60 mg	80 mg
T _{max}	Hr	2.67±1.05	3.50±1.09	3.92±1.08	3.08±0.79
A _{max}	μ g/mL	1.58±0.35	3.08±0.98	5.38±0.75	7.44±1.47
AUC _{0–inf}	μ g*hr/mL	11.79±3.30	32.01±8.84	49.26±8.69	70.76±15.49
T _{1/2}	Hr	4.18±2.21	4.36±1.07	3.70±0.82	3.46±0.86

Note: Pharmacokinetic parameters (±SD) are based on anti-Xa after various enoxaparin sodium doses given subcutaneously in a crossover design in normal subjects. A_{max} is maximum drug activity, T_{max} is time for maximum drug activity and AUC is area under the drug activity versus time curve from 0 to infinity.

Source: Adapted from Ref. 10.

Table 5 Enoxaparin Bioavailability. Pharmacokinetic Parameters^a after 5 Days of 1.5 mg/kg SC Once Daily Doses of Enoxaparin Sodium Using 100 mg/mL or 200 mg/mL Concentrations

	Concentration	Anti-Xa	Anti-IIa	Heptest	aPTT
<i>A</i> _{max}					
(IU/mL or Δsec)	100 mg/mL	1.37 (± 0.23)	0.23 (±0.05)	104.5 (±16.6)	19.3 (±4.7)
	200 mg/mL	1.45 (± 0.22)	0.26 (±0.05)	110.9 (±.1)	22 (±6.7)
	90% CI	102–110%		102–111%	
<i>T</i> _{max} ^b (h)	100 mg/mL	3 (2–6)	4 (2–5)	2.5 (2–4.5)	3 (2–4.5)
	200 mg/mL	3.5 (2–6)	4.5 (2.5–6)	3.3 (2–5)	3 (2–5)
AUC (ss) (h IU/ mL or h Δsec)	100 mg/mL	14.26 (± 2.93)	1.54 (±0.61)	1321 (±219)	
	200 mg/mL	15.43 (± 2.96)	1.77 (±0.67)	1401 (±227)	
	90% CI	105–112%		103–109%	

^a Means ± SD at day 5 and 90%CI of the ratio.

^b Median (range).

Abbreviations: *A*_{max}, maximum drug activity; *T*_{max}, time for maximum drug activity; AUC, area under the drug activity versus time curve; CI, Confidence Interval.

Source: Adapted from Ref. 11.

conducted based on the premise that drugs like formoterol are intended to act locally on the airways. Drug that is available in the systemic circulation after absorption from the lung may not correlate to the therapeutic response in the lung. However, FDA often requires an additional systemic exposure study that measures the concentration of the drug in the plasma after drug administration to the lungs as an indicator of possible toxicity. In cases of locally acting drug products, when a suitable pharmacodynamic endpoint is available, pharmacodynamic BE studies are considered as a better option over pharmacokinetic studies (1,4).

The FEV₁ is used as a pharmacodynamic index for assessing airway obstruction, bronchoconstriction or bronchodilatation. The FEV₁ is the volume of air expired in the first second during maximal expiratory effort. The FEV₁ is used as the pharmacodynamic endpoint for locally acting bronchodilators such as fomoterol and albuterol. The methacholine challenge test is a technique used for testing potential anti-asthmatic drugs, since methacholine mimics the effects of histamine in triggering asthma.

Marzo et al. used a methacholine challenge model to measure the ability of formoterol to maintain the dilation of the airway smooth muscles (12). FEV₁ and PD₂₀ FEV₁ were the pharmacodynamic endpoints used to evaluate differences between the test and reference drug products. A dose escalation study (13) ranging from 12 to 96 μg of formoterol utilizing FEV₁ as the pharmacodynamic endpoint showed that formoterol exhibits a linear effect (Fig. 7). Although Marzo et al. did not perform a dose escalation

study; the selected dose of 12 μg formoterol in their study was between the 20% and 80% of the linear dose response curve and could discern small differences in bioavailability between two drug products. These investigators also reported a cumulative urinary excretion of formoterol in addition to a pharmacodynamic study (Table 6). Table 7 summarizes the individual FEV₁ values obtained for the test and reference formoterol fumarate dry powder inhaler products. Table 6 shows the cumulative urinary excretion (CUE) of formoterol in 24 healthy human volunteers treated with the Test and Reference formulations of formoterol. Table 8 shows the Test/Reference ratio's based on point estimates and 90% CI for both FEV₁ and CUE_{0-12hr} for formoterol fumarate.

The data from the pharmacokinetic approach showed that the 90% CI of 80 to 125 was not met and as such, BE could not be declared. However, using the pharmacodynamic approach for BE, the 90% CI for FEV₁ were

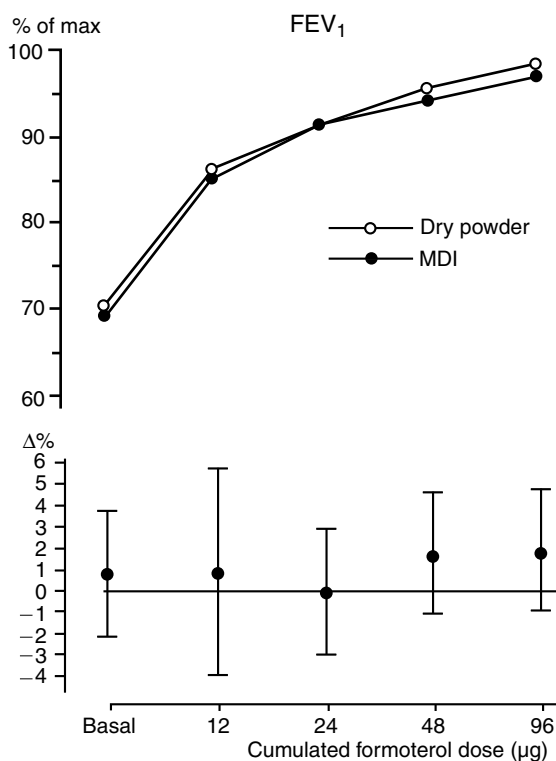


Figure 7 Dose escalation study for formoterol fumarate. FEV₁ (percentage of maximum individual value) obtained after inhalation of increasing doses of formoterol pMDI (solid circle) and DP (open circle). Lower panel shows mean difference with 95% CI for difference. Source: Adapted from Ref. 13.

Table 6 Cumulative Urinary Excretion of Formoterol in 24 Healthy Volunteers Treated with the Drug, Test and Reference Formulations

Subject number	Test		Referecne	
	ng	%	ng	%
1	858.64	8.74	1042.60	10.61
2	749.80	7.63	1135.70	11.56
3	1070.20	10.89	829.30	8.44
4	610.30	6.21	809.26	8.23
5	651.50	6.63	808.00	8.22
6	657.60	6.69	414.66	4.22
7	1234.00	12.56	605.94	6.17
8	647.20	6.59	474.50	4.83
9	818.24	8.33	561.52	5.71
10	105.00	1.07	786.00	8.00
11	775.45	7.89	1183.60	12.04
12	591.40	6.02	379.48	3.86
13	573.70	5.84	968.30	9.85
14	703.00	7.15	716.28	7.29
15	774.20	7.88	441.50	4.49
16	591.22	6.02	556.80	5.67
17	868.50	8.84	887.30	9.03
18	823.04	8.37	1162.10	11.82
19	531.10	5.40	1166.50	11.87
20	1184.00	12.05	1059.60	10.78
21	591.00	6.01	796.88	8.11
22	1146.50	11.67	851.30	8.66
23	1115.00	11.35	785.00	7.99
24	559.50	5.69	1510.22	15.37
Mean	759.59	7.73	830.51	8.45
SD	256.05	2.61	286.97	2.92
CV%	33.71	33.76	34.55	34.56

Source: Adapted from Ref. 12.

within the required intervals and proved to be a better way to establish drug product performance. The interesting observation here is that formoterol marginally fails the BE criterion (using traditional pharmacokinetic approaches. In this case, the variability in the cumulative urinary excretion may have been high and the study might need to be re-designed to improve power of the statistical data.

ALBUTEROL SULFATE

Albuterol sulfate is a synthetic sympathomimetic amine and a selective β_2 -adrenergic bronchodilator. Albuterol is administered either by inhalation or

Table 7 FEV₁ and PD₂₀ FEV₁ Values Measured in the Volunteers Treated with Test and Reference Formulations of Formoterol Fumarate Bihydrate

Subject number	FEV ₁ (L)		PD ₂₀ FEV ₁ (mg)	
	Test	Reference	Test	Reference
1	3.67	3.07	^a	2.564
2	3.32	2.83	^a	5.150
3	1.91	1.88	^a	3.522
4	2.01	2.89	3.431	2.996
5	3.82	4.06	^a	^a
6	2.95	2.86	1.459	2.150
7	3.41	2.61	2.414	1.718
8	5.33	4.90	^a	2.060
9	1.94	1.89	3.337	1.818
10	2.07	2.05	0.873	1.745
11	2.24	2.45	3.636	5.174
12	2.20	2.05	0.647	0.534
13	4.08	3.72	^a	5.924
14	5.06	4.95	^a	^a
15	2.62	3.09	0.929	0.721
16	2.64	2.61	3.263	3.651
17	3.87	3.21	^a	6.306
18	2.84	2.66	^a	^a
19	2.25	2.62	1.927	3.421
20	2.30	2.77	3.827	2.737
21	3.38	3.37	2.183	2.211
22	2.65	4.43	3.722	^a
23	2.90	3.18	4.401	^a
24	4.05	4.36	^a	^a
Arithmetic mean	3.06	3.10		
SD	0.96	0.89		
CV%	31.37	28.71		
Geometric mean	2.93	2.99		

^a Total protection.

Source: Adapted from Ref. 12.

orally for the symptomatic relief of bronchospasm. Reference listed drugs for the aerosol metered dose inhaler (MDI) formulations include Ventolin[®] (GlaxoSmithKline), Proventil[®] (Schering), Proventil HFA (3M), Ventolin HFA[®] (GlaxoSmithKline), and Proair HFA[®] (IVAX RES). Similar to formoterol fumarate, albuterol is administered for local action in the lung and delivery to the site of action occurs prior to systemic absorption. The rate and extent of absorption after inhalation may be difficult to

Table 8 Point Estimator (Test/Reference Ratio of Geometric Means), 90% Confidence Intervals and Results of Crossover ANOVA with FEV₁ and CUE_{0–12 hr} in the Comparative Study with Formoterol Fumarate

	Test/Reference	90% CI	Crossover ANOVA
FEV ₁	0.98	0.92–1.04	<i>P</i> NS
CUE _{0–12 hr}	0.90	0.73–1.11	<i>P</i> NS

Abbreviations: ANOVA, analysis of variance; NS, not significant.

Source: Adapted from Ref. 12.

characterize due to the complex processes involved, including absorption into systemic circulation from highly perfused lung tissues combined with gastrointestinal absorption from the swallowed fraction of the administered dose and the possibility of first pass effects (14). Hence, monitoring a pharmacodynamic endpoint is appropriate for comparative studies of inhaled albuterol formulations.

With the development of various formulations and delivery systems for inhaled drug delivery, there are numerous published studies that include comparisons of drug deposition (in vitro studies) and therapeutic/pharmacodynamic indices (in vivo studies). The FDA has issued draft guidance documents for bioavailability and BE studies of these drugs (4,11).

The development of a suitable pharmacodynamic bioassay for albuterol after inhalation is particularly difficult due to the small and highly variable amount of drug reaching the site of action in the lungs and the probable large intra-subject variability in the response at different times. Pharmacodynamic endpoints in comparative albuterol studies include FEV₁(forced expiratory volume in 1 s) (15), methacholine-induced bronchoconstriction/FEV₁ (16,17), and peak expiratory flow (18). FEV₁, as previously mentioned, is a measurement for assessing BE and this effect has been modeled using an $E_{\max}$ model, which allows for a baseline effect with zero dose and a maximal effect beyond which an increase in dose yields does not result in a further change in response. The administered dose is a concern for application of the $E_{\max}$ model since a single 90 µg actuation of albuterol produces an increase in FEV₁that is close to the peak response (19). The sensitivity in the bioassay may be maintained by selecting a specific patient population that has a linear dose-response relationship over the dose range of interest, reducing the dosage, or monitoring another pharmacologic effect (19).

A methacholine challenge dose-response study has been proposed as a sensitive method for assessing BE of albuterol based on pharmacodynamic endpoints. In the methacholine challenge dose-response study (17), subjects received three placebo doses (1,2, and 4 actuations) and nine different albuterol doses ranging from 9 to 576 µg on each of 12 study days, with at

least 2 days of washout between doses. Baseline FEV₁ was determined at screening. Fifteen minutes after albuterol dosing on each study day, a methacholine challenge was initiated with a dosimeter method at concentrations from 0.078 to 125 mg/mL. PD₂₀, the provocational dose of methacholine required to achieve a 20% reduction in FEV₁ following a dose of albuterol was determined. PD₂₀ values were fitted to an $E_{\max}$ model to determine ED₅₀ (albuterol dose required to achieve 50% of fitted maximal value of the pharmacodynamic effect above baseline), $E_{\max}$ (fitted maximal pharmacodynamic effect above baseline), and E_0 (pharmacodynamic effect at zero albuterol dose/placebo). Results of this study are summarized in Table 9 and 10. In this study, statistically significant differences were not observed between the PD₂₀ values at screening and those after placebo and mean PD₂₀ values after albuterol administration were significantly greater than the values after placebo. One and two actuations yielded responses in the rapidly rising portion of the dose-response curve, less than 80% of $E_{\max}$, and an increase in mean responses was observed when the dose was doubled, approaching a doubling of response. Hence, the dose-response was characterized in the single albuterol dose/methacholine challenge study design, one of the requisites for establishing a pharmacodynamic bioassay for documentation of BE.

Table 9 Methacholine Chloride PD₂₀ Values (Geometric Mean Dose, CV%) at Screening, after Placebo, and after Albuterol Administration

Treatment	Geometric mean	CV (%)
SCR	0.0374	72.0
Placebo 1	0.0568	215.4
Placebo 2	0.0384	121.1
Placebo 4	0.0500	199.7
9	0.1302	104.8
18	0.1562	108.1
36	0.1960	134.1
2 × 36	0.4608	200.1
72	0.2604	250.7
90	0.3097	139.5
180	0.5759	187.7
288	0.6007	124.0
576	1.0003	171.7

Note: Subjects ($n = 15$) received placebo or albuterol followed fifteen minutes later by a methacholine challenge using a dosimeter method; the cumulative dose of methacholine required to achieve a 20% reduction in FEV₁, PD₂₀ (mg) computed from log-transformed data, was determined.

Abbreviations: CV, coefficient of variation; SCR, baseline at screening.

Source: Adapted from Ref. 17.

Table 10 ED₅₀, E_{max}, and E₀ Values from Fitting Methacholine Chloride PD₂₀ Values Acquired in an Albuterol-Methacholine Bronchoprovocation Study to an E_{max} Model

Parameter	Mean (μg)	SD (μg)	CV (%)
ED ₅₀ ^a	119.2	—	69.0
E _{max}	1941	2846	147
E ₀	97.1	98.0	101

Note: Individual PD₂₀ values (see Table 9 for geometric mean data), the cumulative doses of methacholine required to achieve a 20% reduction in FEV₁ at screening, after placebo, and after albuterol administration were fitted to an E_{max} model that included a baseline response, $E = E_0 + \frac{E_{max} \cdot Dose}{ED_{50} + Dose}$, in this model, the pharmacodynamic effect (E) is PD₂₀.

^aGeometric mean and CV for ED₅₀ computed from log-transformed data (no SD reported); arithmetic mean reported for E_{max} and E₀.

Abbreviation: CV, coefficient of variation.

Source: Adapted from Ref. 17.

APPLICATION OF A BRONCHOPROVOCATION MODEL

Stewart et al. (20) used histamine bronchoprovocation and a bioassay statistical procedure to evaluate the in vivo BE of a generic albuterol MDI compared to the RLD, Ventolin (GlaxoSmithKline). In a randomized, crossover study including 24 subjects with mild to moderate asthma, one treatment was administered on each of 4 study days (one and four actuations, 90 and 360 μg). A histamine bronchoprovocation procedure was initiated 1.25 hours before and 15 minutes after administration of the study treatment. The primary pharmacodynamic measure was the provocative concentration of histamine causing a 20% decrease in FEV₁. After study treatment administration, there was a significant dose-effect relationship ($P < 0.0001$). Differences in the overall mean response between the two formulations ($P = 0.68$) were not significant. It was determined that one actuation of the generic albuterol MDI was equivalent to 1.01 puffs of Ventolin (90% CI, 0.69–1.50). The authors concluded that the generic albuterol MDI delivered a quantity of albuterol to the β₂-receptor site in the lung that was bioequivalent to Ventolin. Note that the range of 67% to 150% was previously considered by the FDA for the approval of generic albuterol MDIs. This criterion is stated in review of ANDA 73-045 which is available on the FDA internet website or through freedom of information (FOI).

In another example, a randomized two-treatment, four-period, two-sequence crossover replicate design study was used to compare a test formulation to Ventolin Inhalation Aerosol (FOI; review of ANDA 73-045). Each study day consisted of a pre-albuterol methacholine challenge followed at least three hours later by administration of the assigned albuterol treatment and a post-albuterol methacholine challenge. Twenty-five patients

with mild to moderate asthma completed the study. The methacholine PD₂₀ measured after the test formulation was compared to the PD₂₀ after treatment with the reference product. The ratios of the post-albuterol PD₂₀ to the pre-albuterol PD₂₀ for each treatment were also compared. The results of this study are shown in Table 11.

Based on statements in FDA guidance documents and published review articles (19), therapeutic effectiveness of albuterol delivered by MDI is dependent in part on the in vitro performance of the drug delivery system. FDA, Office of Generic Drugs is requesting both in vitro and in vivo data to document BE between a generic and the reference listed drug product given by MDI. In vitro data serve to characterize the test product during development and serve a post-approval quality control function. Although in vitro tests have been established for MDI products, requirements for in vivo documentation of BE are less well defined. Assuming that validation data can be produced, the FDA believes that a pharmacodynamic endpoint study will be the most suitable method to document BE between different albuterol MDI drug products.

TOPICAL CORTICOSTEROIDS

Topical corticosteroids, such as hydrocortisone, are often applied to the skin in the form of ointments and creams for local activity. A pharmacodynamic approach based on the Stoughton-McKenzie vasoconstrictor bioassay has been used for the determination of BE of these drugs (FDA Guidance for Industry: Topical dermatologic corticosteroids: in vivo BE, June 1995). The

Table 11 Statistical Analysis of Methacholine Chloride PD₂₀ Values from a Replicate Pharmacodynamic Bioequivalence Study of Albuterol using Methacholine Bronchoprovocation

Measurement (Log scale) ^a	Test LS mean	Reference LS mean	Ratio (T/R)	90% CI	Significance ($\alpha = 0.05$)	Power (%)
Pre-albuterol PD ₂₀	-2.65715 (0.070)	-2.55713 (0.078)	0.90	0.79 – 1.03	NS	71
Post-albuterol PD ₂₀	-1.15123 (0.316)	-0.94227 (0.390)	0.81	0.68 – 0.98	NS	<50
Post-/pre- albuterol PD ₂₀	1.50592 (4.508)	1.61487 (5.027)	0.90	0.73 – 1.10	NS	<50

^a Comparison of least squares mean PD₂₀ values based on log-transformed data, pre-dose and after administration of a test formulation and reference product (Ventolin); mg of methacholine (anti-log values) required to produce a 20% decrease in FEV₁ are shown in parentheses; $n = 25$.

Abbreviations: CI, confidence interval; NS, not significant.

Source: Adapted from Ref. 10; review of ANDA 73-045.

vasoconstrictor assay also known as the skin blanching assay and the use of this assay for determination of BE is included as a chapter in the Specialty Drug Product Book (in preparation).

SUMMARY

A BE study may be considered as an *in vivo* measure of drug product performance. The most objective approach to the measurement of bioavailability is the measurement of the drug concentrations in biological fluids such as serum or plasma. The use of drug concentration measurements in blood presumes that there is a relationship between the plasma drug concentrations and drug safety/efficacy. Hence, the measurement of plasma drug concentrations in BE studies can be considered as a surrogate biomarker for *in vivo* drug product performance.

Pharmacodynamic endpoints can be used to determine the BE of drug products when the active drug cannot be measured *in vivo* by a direct physical-chemical assay. The variability of the pharmacodynamic response is generally greater than the variability observed for the measurement of drug concentrations in biologic fluids. Therefore, meeting BE criteria of 90% CI between 80% and 125% on a log-transformed basis is more difficult using pharmacodynamic endpoints.

For pharmacodynamic studies, the drug response versus time profile may not be the same as the drug concentration versus time course. Pharmacodynamic endpoints can be used for BE studies as long as the drug response versus time profiles reflect changes in bioavailability of the drug from the drug product. When performing a pharmacodynamic study, the drug dose administered and the resulting drug response should be close to the ED_{50} so that a change in drug bioavailability is reflected in a change in drug response. If the drug response is at or near the maximum drug response (E_{max}) then small changes in drug bioavailability will not be observed using a pharmacodynamic endpoint.

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Bioequivalence Using Clinical Endpoint Studies

Christopher Hendy

Novum Pharmaceutical Research Services, Pittsburgh, Pennsylvania, U.S.A.

INTRODUCTION

Although much has been written concerning the assessment of bioequivalence using systemic absorption, bioavailability assessments of non-systemically absorbed drug products, or products whose therapeutic site of action may be localized to the site of application, have been minimally addressed in regulatory guidances and elsewhere in the literature.

In the United States of America, the Code of Federal Regulations (CFR) describes the types of studies that are suitable for demonstrating bioequivalence (21 CFR Part 320) (1). The recommendation is that in-vivo clinical comparative bioequivalence studies should be considered only when measurement of the active ingredient in biological fluids, or the use of a pharmacodynamic response, are impossible or not relevant. Other regulatory authorities such as the European Agency for the Evaluation of Medicinal Products, Health Canada's Therapeutic Products Directorate and Japan's Organization for Pharmaceutical Safety and Research have proposed a similar hierarchical structure for selection of study designs (2–4).

For oral or other formulations intended for systemic action, standard pharmacokinetic calculations such as area under the plasma curve (AUC) and peak plasma concentration ($C_{\max}$) can be consistently used across all studies. In clinical endpoint studies, the actual parameters to be used to determine bioequivalence are both product and formulation specific. With few exceptions, there is limited regulatory guidance for these types of product.

It is beyond the scope of this chapter to provide specific details on the study design and analysis for every such product. Rather, this chapter will provide a general overview of the type of products for which a clinical endpoint study may be necessary and of the basic guidelines and principles of protocol design and data analysis. Hopefully, this overview will stimulate further discussion between all interested parties on this rapidly growing and important area of generic drug development.

WHEN IS A CLINICAL ENDPOINT STUDY APPROPRIATE?

Before deciding which type of in-vivo testing may be most appropriate for any generic drug formulation, it is worthwhile to reflect on the primary objective of bioequivalence testing. A generic pharmaceutical is considered acceptable only if the patient can be guaranteed that it is comparable to the brand name equivalent. That is, the generic formulation can not be better, or worse, than the already approved product in terms of its pharmacological/clinical effects. Therefore, the primary objective of any bioequivalence testing is to focus on comparing the performance of the test formulation to that of the reference standard.

For oral formulations intended for systemic action, the measurement of the circulating active ingredient is the most sensitive and relevant methodology. Because only a few physiological steps are involved, and only two of these are usually formulation dependent—namely; dissolution of the formulation followed by absorption through the gastrointestinal (GI) wall—then in most cases both the inter and intra-subject variability is low and the data set (i.e., subject number) can be relatively small. The use of each subject as their own control, either through a two-way cross-over study or a replicate study design further minimizes the variability of the data set. It is also important to recognize that oral dosage forms are unit dosed, and all participants in a pharmacokinetic bioequivalence study will receive an identical amount of the test and reference drug. These studies are also usually performed in a very controlled environment, where procedures are in place to minimize extraneous activities (e.g., water consumption, excessive exercise) that may directly influence the rate of dissolution/absorption of the formulation.

When additional physiological steps are involved before a pharmacological response can be identified or many days of dosing in an uncontrolled environment are required before a positive clinical effect can be measured, both the accuracy and sensitivity of determining true formulation differences is decreased, while the variability is increased.

In many clinical endpoint studies the drug product (for example a cream or ointment for dermatological use) is often not unit dosed. The amount of active ingredient within daily applications and between patients can be quite different. To evaluate the clinical response, the study may last for many weeks while the patient is outside of the investigators direct

control. Finally, a cross-over design is rarely feasible for clinical endpoints and thus the inter-subject variability, which is always significantly greater than intra-subject variability, determines the subject numbers needed.

For accuracy, sensitivity, ease of study conduct, cost and timing, a pharmacokinetic endpoint is normally preferable to a pharmacodynamic or clinical endpoint. An example decision tree that may be useful in determining what type of study may be appropriate for any particular product is shown in [Figure 1](#).

TYPES OF FORMULATIONS REQUIRING CLINICAL ENDPOINT STUDIES

Most discussion of clinical endpoint bioequivalence studies has focused on topical products used to treat dermatological diseases (e.g., creams, ointments and gels). However, there are many other types of drugs whose primary mode action may be considered local or where systemic blood levels may not be appropriate as stand alone evaluation of bioequivalence.

Topical Products

Topical Products for Localized Skin Disorders

With the exception of the topical corticosteroids, where a pharmacodynamic vasoconstrictor assay has been allowed in the United States and Canada to demonstrate bioequivalence, most topical products are indicated for the treatment of diseases associated with some type of localized infection or skin disruption, whose etiology can be diagnosed by a trained physician (5).

In the case of fungal diseases such as *Tinea pedis* (athletes foot) and *Tinea corporis*, infections may occur on the outermost areas of the skin, and the effectiveness of the study formulation can be evaluated using both clinical observation and microbiological identification and culture.

For diseases such as acne or rosacea, the disease is manifest by clinical signs such as inflamed/noninflamed lesions, erythema, and telangiectasia. Efficacy can be evaluated by measuring the effect of the study drug on reducing the severity of certain symptoms, such as inflamed lesion count, over a pre-determined duration of treatment.

The clinical effectiveness of topical products used for the treatment of psoriasis and eczema have traditionally been evaluated using physician scales, which rate the extent and severity of a number of signs and symptoms of each disease being studied. These are often combined into a single severity rating score such as the Psoriasis Activity Severity Index (PASI) or a Physician Global Assessment (PGA) (6,7).

Treatment for cancerous and pre-cancerous skin conditions such as squamous and basal cell carcinoma or actinic keratosis can only be

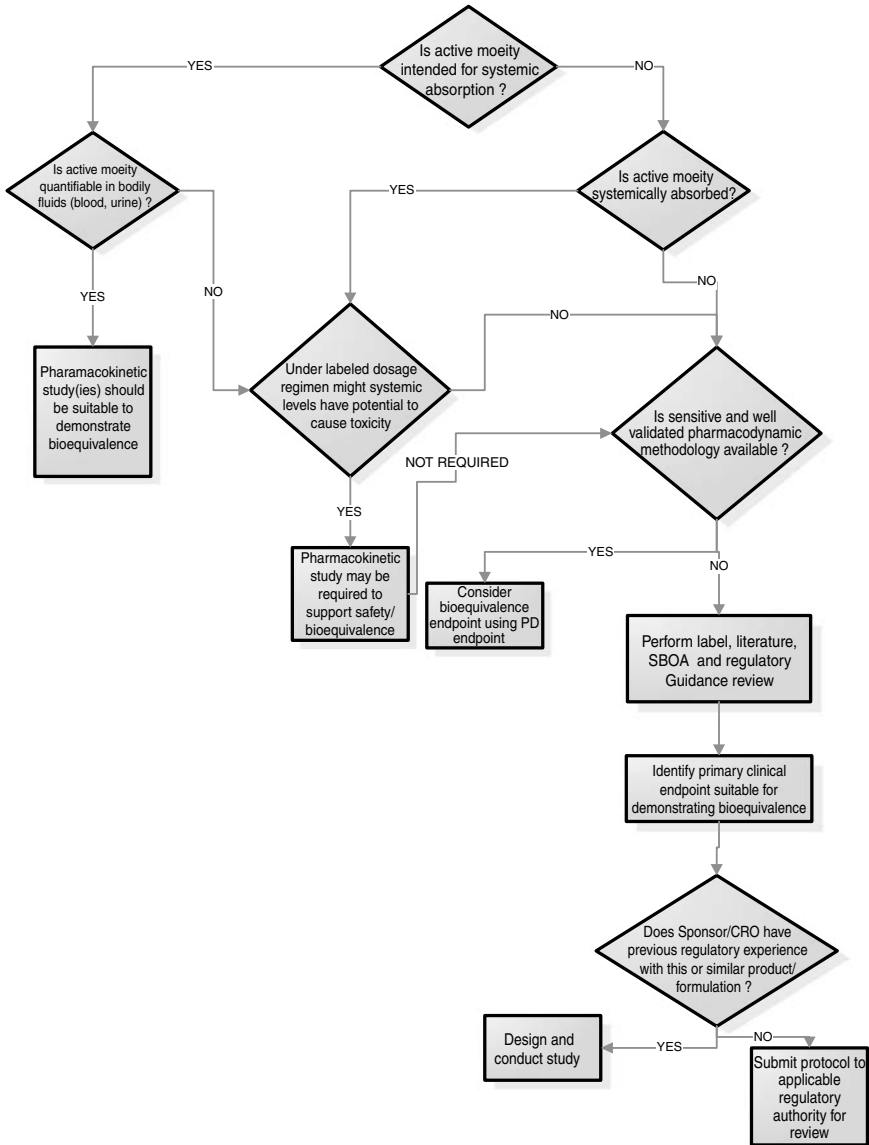


Figure 1 A sample decision tree to determine whether a clinical endpoint bioequivalence study may be required.

considered successful if they completely eradicate the lesions. In such a study, the proportion of patients showing a 100% cure rate could be considered a suitable endpoint (8–10). Similar assessments can be applied to products intended for the treatment of less serious but painful and

cosmetically unpleasant eruptions such as cold sores and warts. In these cases the time to cure is also important to the patient and must be considered when comparing a generic formulation to the reference standard. For miscellaneous products such as depigmentation agents, wrinkle-reducing lotions, prescription antiperspirants and deodorants, the study specific endpoints should be identified using the reference product labeling and literature review.

Topical Products for Treatment of Secondary Infections

Skin infections often manifest themselves secondary to other traumatic dermal events such as cuts, abrasions or surgery. In nonserious cases, these are usually treated with topical antibiotics (such as mupirocin) and endpoints could include a combination of mycological testing and a clinical evaluation.

Nasal Sprays and Inhaled Products Intended for Local Site of Action

Anti-inflammatory drugs such as inhaled albuterol or steroidal nasal sprays (e.g., fluticasone, mometasone) act directly on the inner lung or nasal mucosa and should be considered locally acting. The use of a pharmacodynamic response that is recognized to be linked to clinical symptoms, such as the forced expiry volume (FEV) of the lungs in asthma patients, may provide a useful endpoint (11,12). In other situations where no true pharmacodynamic response has either been validated or is easily measurable, as in the case of topical nasal sprays used to treat seasonal allergic rhinitis (SAR), the relief of the patient's signs and symptoms should be measured.

In both the above-mentioned cases, the active drug moiety is also systemically absorbed, either because much of the product is actually swallowed or absorbed through the local site of action. It is important to recognize that a generic formulation must not only be clinically equivalent to the reference product, but it must also have a similar safety profile. In cases where the active ingredient is also absorbed, and there is potential for systemic toxicity, the clinical study may also need to be supported by a pharmacokinetic study for comparative safety rather than for efficacy purposes.

Optic and Otic Products

If a topical product applied to the eye or in the ear is intended to treat an infection, then the same principles that apply to dermatological anti-infectives may be considered, and a dichotomous clinical endpoint of clinical cure or clinical failure may be used.

For formulations where treatment is intended to bring relief of symptoms rather than a complete cure [e.g., ophthalmic preparations used for reducing intraocular pressure (IOP)], a comparative study between the two formulations to compare effectiveness in reducing IOP may be feasible.

Rectal and Vaginal Products Not Primarily Intended for Systemic Absorption

For anti-infectives the same endpoints as described earlier may be utilized. If the product is systemically absorbed, then a pharmacokinetic study for safety purposes may also be required. It is important to evaluate each product individually in such cases. As an example, although metronidazole 0.75% vaginal gel results in plasma levels that are approximately 2% of those seen after administration of an oral tablet, they are sufficiently high that the potential for nonlocally acting adverse events cannot be eliminated (13). In contrast, when the antibacterial clindamycin is applied locally to the vagina as a 2% cream, the plasma absorption of this drug is extremely low, and there are no concerns about possible systemic toxicity (14). Both products are indicated for the treatment of bacterial vaginosis.

There are several vaginally applied hormonal products indicated specifically for the treatment of signs and symptoms of vaginal atrophy such as dryness, itching and scratching. These products are not intended to treat all of the symptoms of peri or post menopause, such as hot flashes or sweating. Clinical equivalence can be assessed by evaluating changes in vaginal cell cytology using standard markers such as the Vaginal Maturation Index and vaginal pH (15). Although intended for local application, use of these products can lead to significant systemic absorption of estradiol. A supporting pharmacokinetic study for safety purposes should also be considered.

Oral Products that Act Locally within the GI Tract or that Are Not Systemically Absorbed

For drug products intended to treat diseases of the GI tract and infections (parasitic worms), that are not systemically absorbed, or where plasma concentrations do not correlate with clinical efficacy, then clinical endpoint studies may be required.

Sucralfate, which coats active duodenal ulcers and protects them from direct contact with acidic gastric juices, has minimal systemic absorption. Several generic formulations of sucralfate have been approved in the United States. These studies have employed endoscopic evaluations to compare the ability of the generic and reference formulations to heal duodenal ulcers (16).

Mebendazole is a broad spectrum antihelminthic that acts directly on the infecting parasitic worm. Accordingly, circulating blood levels in humans are irrelevant to this drug's mode of action, and thus, its potential efficacy. A study comparing worm eradication rates would confirm clinical equivalence.

There are currently a number of formulations of the anti-inflammatory agent mesalamine approved for marketing. These include rectal suppositories, an enema suspension and controlled release tablet and capsules.

Mesalamine appears to act directly on the GI tract by reducing prostaglandin production and therefore reducing inflammation in diseases such as inflammatory bowel disease/ulcerative colitis. Several generic mesalamine suspensions have been approved on the basis of systemic pharmacokinetic data. However, the Food and Drug Administration (FDA) have requested that generic applications for solid dosage forms include clinical endpoint studies in addition to possible pharmacokinetic studies.

VARIABLE VERSUS DICHOTOMOUS ENDPOINTS

The primary clinical endpoint for any study is the most critical component of the study design as it will ultimately be used to determine bioequivalence and to validate the sensitivity of the study.

The type of endpoints that may be used fall into two very distinct categories.

Variable Endpoints

Continuous Open Ended Rating Assessments

Continuous endpoints are those that directly measure a change from baseline in some aspect of the disease being treated. The strongest of these types of endpoints rely on direct, noninterpretive measurements of the disease state, such as change in inflamed and noninflamed lesion count from baseline in patients with acne or rosacea. When these assessments are performed by appropriately qualified, trained, and validated evaluators, their non-interpretive nature can provide extremely reliable data sets, with low intra and inter-subject variability. In cases where observations for all patients can be made by a few individuals the variability can be reduced significantly.

This type of data set is also used to investigate superiority to placebo or a comparator product and has been used extensively by medical regulatory reviewers to support New Drug Applications. However the analysis of such data for bioequivalence can be difficult. These rating scales are usually closed at one end but open at the other. Accordingly, two sided comparative analyses, used for true analysis of bioequivalence, can be difficult as the data set is usually highly skewed in one direction (17). For example; if the primary endpoint in an acne study is percent reduction in inflamed lesion count, then no patient can show more than a 100% reduction if all of their inflamed lesions are cleared at the end of the study. However, as there is no closed upper end point, subjects whose inflamed lesion counts increase during the study can significantly skew the data set. The dangers of looking at the traditional mean Test/Reference ratio for such data sets are apparent. Just one or two patients who show a significant worsening of the disease with the reference drug can severely impact this ratio, and the test product may not meet the usual 90% confidence interval

(CI) criteria for bioequivalence due to an anomalous result in the reference treatment group.

Closed or Categorical Scales

In most cases where a graded response is utilized, either the patient or an appropriately qualified individual can categorize the signs and symptoms of their disease using pre-determined rating scales. In a study evaluating the clinical efficacy of a topical nasal steroid intended to treat the signs and symptoms of allergic rhinitis (AR), the primary endpoint is usually based on the patient's assessment of four symptoms such as nasal congestion, runny nose, sneezing, and itching. (18). For psoriasis, the investigator may be required to make assessments of scaling, plaque elevation, or erythema of the infected areas (6,7). When multiple signs or symptoms occur, these are often more meaningful when combined to give a total score rather than looking at changes in individual symptoms. The sum of all four scores for a patient's assessment of nasal allergy can, therefore, be combined to produce a Total Nasal Symptom Score (TNSS). To minimize inter and intra-subject variability, the various grading of responses must be as well defined as possible, such that interpretation between investigators and/or study participants is reduced (see section "Reducing Variability").

These scales are most effective when designed and used as static assessments rather than reflective ones. This requires that the rating for each assessment for a particular patient must be a stand alone evaluation, based only on the disease state at the time the assessment was made. The use of reflective scoring, which requires the investigator or patient to determine how the signs and symptoms may have improved or worsened since baseline or a previous visit is extremely subjective. This type of scoring often requires the person involved in the rating to remember the severity of the disease for the previous 12 weeks (or more) and the data can be easily manipulated.

Categorical scales, while more subjective than direct measurable observation, have some advantages with respect to data handling in as much as they are closed at both ends, and there are a limited number (usually 4–6 severity ratings) of outcomes. Thus the effect of individual outliers or aberrant results on mean values is not as dramatic as open ended scales are. However, because of the limited scoring possibilities, they are also less sensitive to differences between two products that are not bioequivalent.

Dichotomous Variables

By definition, dichotomous variables have only one of two outcomes. As with continuous variables, these can be based on very specific, non-interpretive end points such the presence or absence of *Tinea pedis* infection by mycological culture or by some minimum score on a PGA, which would define a therapeutic cure. In several cases a combination of two or more

outcomes may be used to give an overall therapeutic cure rate. *Tinea pedis* patients can be considered a “therapeutic cure” if they have a negative mycological culture (“mycological cure”) and are considered a “clinical cure” using the investigators grading of signs and symptoms.

The use of dichotomous endpoints solves the problems of skewed data sets. On condition that the 90% CI of the proportion of subjects in the test group who are considered a “cure” compared to the proportion of subjects in the reference group who are considered a “cure” falls within the range ± 20 then clinical equivalence can be considered to have been demonstrated.

CHOOSING APPROPRIATE CLINICAL ENDPOINTS

In designing a study protocol aimed at demonstrating bioequivalence, the choice of which endpoints to use is often the most critical step that will affect the outcome of the study. Again it is worthwhile reminding oneself that the primary objective of the study is to identify any differences in the test formulation that may result in it being considered bioinequivalent to the reference standard.

Resources for Determining Endpoints

Labeled Indication

Regulatory authorities require that marketed generic drug products carry the same patient labeling as the reference listed product (RLD). The first step in determining the clinical endpoint to be utilized should be to carefully examine the approved labeling of the RLD. How critical this is cannot be overstressed. For example, in the U.S. mupirocin 2% cream is indicated for the treatment of impetigo, yet the 2% ointment is indicated for the treatment of bacterial infection secondary to wounds (19,20). Therefore, if the incorrect endpoint is chosen then, not only would the study be inappropriate, but the Study Sponsor, Clinical Research Organization managing the study, and the investigators could face severe regulatory penalties for conducting a clinical study for an off-labeled indication without appropriate approval. Further, the labeled indications for the same product in different countries may be quite different. The prescription formulation of the anti-fungal product ketoconazole 2% shampoo is labeled in the United States for the single dose treatment of *Tinea versicolor*. In Canada and the United Kingdom it is available over the counter for the multiple dose use in the treatment of seborrheic dermatitis.

It is important to note that the signs and symptoms within the disease category should also be scrutinized. For example, product labeling for different topical antiacne products can carry quite different indications with regard to the type of skin lesions associated with acne vulgaris. The labeling

for BenzaClin[®], a combination cream product containing the antibacterial agent 0.1% clindamycin and 0.5% benzoyl peroxide is indicated for the treatment of the inflamed lesions of acne, whereas sulfacetamide 0.1% lotion is indicated for both the treatment of inflamed and non-inflamed lesions (21,22). Thus the endpoints that should be used to determine bioequivalence may be different for these two products, even though they are indicated for the same disease. Similarly, Flonase[®] (fluticasone) 0.1% nasal spray is specifically indicated for the treatment of the nasal signs and symptoms of SAR and perennial allergic rhinitis but not for ocular symptoms such as red, watery or itching eyes. Including the latter symptoms in a clinical endpoint bioequivalence study for fluticasone would provide no useful additional information on the generic formulation (23).

Studies Conducted by the Innovator

The Freedom of Information Act in the United States requires that following approval of a new chemical entity, a summary of the basis of approval document (SBOA) be made available to the general public and other interested parties. While confidential and trade secrets are redacted from these documents, summaries of the clinical studies conducted as part of the new drug application (NDA) for the innovator are usually described in some detail. These can provide useful information in determining the appropriate clinical endpoints that were used in the approval process of the innovator product. They can also detail the length of treatment, degree of clinical effect seen, any known placebo effect, basic inclusion/exclusion criteria and other factors that may be important in study design. Many of these SBOAs can be accessed on line through the FDA website (24) or by writing to the FOI office and requesting a copy of the relevant SBOA. The SBOA for already approved generics may also be available from the same sources.

Literature Review

The development of online search engines has made literature review much simpler over the last few years. As many of the drug products that are being considered for development by generic manufacturers have been extensively researched, the amount of published material can be quite significant. For example a PubMed search of metronidazole and rosacea generated over 172 hits including over 20 peer-reviewed papers that provide very detailed designs and results of both placebo and comparative clinical endpoint studies using metronidazole 0.75% topical formulations.

Published Regulatory Guidance

Many national regulatory authorities have published detailed guidelines on the requirements for the design, conduct and analysis of bioequivalence studies with solid oral dosage formulations. However, there is extremely

limited assistance on the requirements for nonoral dosage forms. The reason for this would appear to be twofold. First, only a small proportion (less than 5%) of all abbreviated new drug applications submitted to the FDA for review require a clinical endpoint study evaluation. Second, the requirements and concepts for pharmacokinetic bioequivalence studies are essentially the same for all drugs and formulations and thus can be adequately covered in several general Guidance documents. As should already be clear, the wide range of drugs, formulations and disease states that may require clinical endpoint studies would be impossible to cover in just a few regulatory Guidance. Although there has been progress in the development of specific Guidance for certain products, there is extremely limited information available on study specifics for clinical endpoint bioequivalence studies.

There are a number of Guidances available for the development of new drugs in specific areas, such as acne and AR (18,25). Although these provide useful background material, it must be remembered that the intended audience is very different than the generic developer. These should read with caution and in context of the objective of the clinical endpoint bioequivalence study. The FDA Guidance on developing new drugs for AR for example, recommends that the ocular symptoms also be evaluated. Indeed this is most appropriate for a company trying to develop a new product and hoping to differentiate itself from its competitors by having this in the product label (18). The FDA Guidance on development of generic nasal sprays for AR focuses on the nasal symptoms as most already approved products are labeled only for this indication (see section “Labeled Indication”) (25).

Products with Multiple Indications

Often topical anti-infective products may have more than one indication. Clotrimazole topical products are indicated for the treatment of several strains of the fungus *Tinea* spp. such as *T. pedis* (athletes foot), *T. versicolor* (body fungus), and *T. corporis* (jock itch). As the objective of a clinical endpoint study is to test the formulation, not the efficacy of the active ingredient, testing all the indications would appear to be unnecessary. Demonstrating clinical equivalence to one of the indications would be sufficient to confirm that the test and reference formulation were suitably similar to be considered equivalent. Therefore, the final choice of clinical endpoint in such a case should be driven by additional factors such as the efficacy of the reference product (and placebo if required) in the target disease, the sensitivity of the endpoint in testing the formulations, the number and availability of patients required, the duration of treatment/study and the type and costs of the tests required in the study.

An interesting case study from both a study design and a regulatory perspective involves the topical cream imiquimod (Aldara® 5% cream).

Imiquimod is an immune response modifier that despite having no direct antiviral activity *in vitro* was found to be very effective in the treatment of genital warts. The product was first marketed for this indication only in 1997. In 2004, additional indications of actinic keratosis (AK) and superficial basal cell carcinoma (sBCC) were approved by the U.S. FDA. The mechanism of action of imiquimod is unclear, and no common mode of action has been found in these three diseases. Certainly the location of the disease and the applications site of the cream (i.e., anogenital region for warts, skin and face for AK and trunk, neck, and extremities for sBCC) is significantly different between the three indications. Further, the cellular site of action would also appear to be quite different for these three indications.

The FDA has recommended that a clinical endpoint study for generic imiquimod be conducted in AK patients. Because of the different skin types and disease morphologies of the three indications, the use of a single clinical endpoint study to cover all three indications is debatable.

The issue is further complicated by the fact that imiquimod is currently approved for only genital warts and AK in Canada and only for genital warts and sBCC in the United Kingdom. Obviously the FDA recommended study in AK patients would not be acceptable for the United Kingdom where this is not a labeled indication. For a generic sponsor seeking to obtain approval in United States, Canada and the United Kingdom with the same formulation, a five arm study comparing the same test formulation with the reference product from all three countries and placebo in the indication of sBCC would seem to be much more efficient in terms of time and resources than conducting three different studies in different indications to satisfy local requirements. Further, because the complete clearance rate (treatment success) is higher in sBCC (approximately 75%) compared to AK (approximately 45%), then significantly less patients would need to be enrolled in a study in sBCC than AK (see section “Patient Numbers”).

Products Containing More than One Active Ingredient

Although not as common as with oral dosage formulations there are several topical products that contain two or more active ingredients. With combination oral dosage formulations, these are simply measured by evaluating both analytes in a pharmacokinetic study. This is much more problematic with topical combination products when both products may play a role in the overall clinical efficacy. Both BenzaClin and Benzamycin are topical antiacne products containing a known antibacterial (clindamycin and erythromycin, respectively) and benzoyl peroxide. The latter is considered to have mild antibacterial activity, but its inclusion as an active ingredient in the finished dosage form is primarily to enhance the action of the other ingredient in reducing inflamed lesion count. Therefore, a single clinical study that adequately tests the formulation, as failure for either

ingredient to perform as anticipated will result in a test formulation that is bioinequivalent. On the other hand, a single clinical study would not be sufficient for a product such as Lotrisone[®], a combination cream containing the antifungal clotrimazole and the anti-inflammatory corticosteroid bethamethasone dipropionate. This combination formulation eradicates the infecting organism (*Tinea pedis*) with clotrimazole, and betamethasone relieves the signs and symptoms of associated inflammation such as itching and erythema. In this particular example, a clinical endpoint study to evaluate the clotrimazole action on eradication of the fungus and a vasoconstrictor pharmacodynamic study to confirm equivalence of the corticosteroid component, could be conducted to adequately characterize both active ingredients in the formulation.

Primary and Secondary Endpoints

Clinical endpoint studies rarely contain only one clinical endpoint. Although it is recommended that the most clinically relevant endpoint is designated as the primary endpoint, data on supplementary endpoints or secondary endpoints are also collected during the study. Analysis of this data can provide important supportive evidence of both bioequivalence and they can help to validate the sensitivity of the study.

The Office of Generic Drugs (OGD) has recommended that, for generic topical products intended to treat the inflamed lesions of acne, the mean reduction in the inflamed lesion count from baseline be the primary endpoint on which determination of bioequivalence is evaluated. Although the reference drug may not specify that the product also reduces noninflamed lesion count, most of the currently marketed products do exhibit a favorable reduction on noninflamed lesion count. Therefore, including an assessment of these types of lesions should be incorporated into the study design. While it may not be necessary to demonstrate statistical equivalence to the reference product for this secondary endpoint, certainly the test product would be expected to demonstrate noninferiority. In addition, the inclusion of an assessment of clinical cure using a PGA (Table 1) score can provide important data. The definition of a clinical cure should be clearly identified in the study protocol and statistical plan. In this specific example a reduction of at least two rating points from baseline, could be an a priori definition of clinical cure. This study would enroll only patients with moderate to moderately severe acne (a score of 3, 4 or 5 at baseline).

In a study with a topical product for a fungal disease the primary endpoint (therapeutic cure) could be a combination of a mycological and PGA. These two secondary endpoints could act as stand alone analysis of mycological cure and clinical cure.

Determination of bioequivalence of oral dosage forms focuses on analysis of AUC and $C_{\max}$. Although $T_{\max}$ is reported as part of the

Table 1 An Example of a PGA Rating Scale That May Be Used in the Assessment of Acne

0	= Normal, clear skin with no evidence of acne vulgaris
1	= Skin almost clear, rare non-inflammatory lesions present, with rare non-inflamed papules (papules may be hyperpigmented, though not pink-red)
2	= Some non-inflammatory lesions are present, with few inflammatory lesions (papules/pustules only, no nodular lesions)
3	= Several to many comedones and papules/pustules only and there may or may not be 1 small nodular lesion. Non-inflammatory lesions predominate, with multiple inflammatory lesions
4	= Many inflammatory lesions and many comedones and papules/pustules. There may be a few nodular lesions
5	= Highly inflammatory lesions predominate; variable number of comedones, many papules/pustules and nodular lesions present
A patient who shows a reduction of two or more points on the PGA between their baseline visit and their last evaluable visit will be considered a “clinical success.”	
A patient who does not show at least a two point reduction in the PGA score between baseline and their last evaluable visit will be considered a “clinical failure.”	

Abbreviation: PGA, physicians global assessment.

analysis, it is considered to be important only in certain instances. For example in analgesics intended for fast relief, this parameter would be considered important if the test formulation failed to act in a similar fashion to the reference product. When the equivalence of a formulation is being tested in clinical endpoint study and where time to cure may be clinically relevant, a comparison of the time course of time to cure maybe very important, and should be considered in the choice of endpoints and statistical analysis.

As discussed earlier, imiquimod is approved for the treatment of genitoanal warts and the labeling allows for up to 16 weeks of treatment for this use. Nevertheless, the patient is advised to discontinue use after successful eradication of the warts due to the undesirable side effect profile of this product. Review of the SBOA reveals that, in fact, most patients obtain 100% cure by about the eighth week of treatment. A study employing a clinical cure rate at Week 16 as its primary endpoint would be expected to miss an important assessment of the test formulation, namely the average or median time to complete cure. Therefore, such a study should include an analysis of time to cure for both products as secondary endpoints. A test formulation that fails to show a time to cure rate comparable to the reference product should not be considered bioequivalent.

Secondary endpoints can be over used if they do not provide data that are useful or relevant to the objective of the study. The analysis of each of the four individual symptoms that comprise the TNSS in SAR studies, for

example, would seem unnecessary. Not only are each of the four components inherently linked, by being relieved by the same pharmacological action, but none are sufficiently sensitive enough as stand alone measures to differentiate between formulation differences (26).

STUDY DESIGN

Once the primary and secondary endpoints have been identified and agreed upon, the actual design of the study can proceed. There are multiple principles that must be considered during this process. Some of the most important are considered below.

Use of Placebo Treatment Arm

The risks and benefits to patients enrolled in clinical research studies that include a placebo treatment group must be carefully considered based on the following three criteria: (i) the disease being treated; (ii) the availability, efficacy and safety of already approved therapies; (iii) the scientific and statistical requirements of the desired outcome of the research study. The Office of Human Rights Protection, a Division of the U.S. Federal Government's Department of Health and Human Services, has issued a detailed guidebook to Institutional Review Boards (IRBs) that includes discussion on the use of placebo treatment groups in clinical studies (27).

While the use of a placebo treatment arm has been considered the "gold standard" in clinical study design for many years, the scientific value and ethical use of placebo treatment groups in clinical studies is coming under increasing global scrutiny. The use of a placebo treatment group in comparative clinical endpoint studies must, therefore, be carefully considered for each study. As previously discussed, clinical endpoint studies are generally considered the least sensitive method of determining bioequivalence, and it is important that the study reviewer is confident that the clinical endpoint employed has sufficient sensitivity to differentiate between two formulations that are in fact bioinequivalent. In pharmacodynamic studies designed to assess bioequivalence, the first requirement is to develop a dose relationship model—usually a sigmoidal type response curve. This enables identification of doses on the ascending portion of a dose response curve, which can be used in the design of pivotal studies. It is often impossible to build such a dose response curve for clinical endpoint studies since the RLD is only marketed at a dose that generates the maximal therapeutic response. In such cases, showing that both the test and reference product have demonstrated a superior therapeutic response compared to a placebo provides some reassurance that a potential generic formulation is not sub-potent to the RLD.

The OGD of the FDA have determined that in order to confirm the sensitivity of a clinical endpoint study to differentiate between two possibly

bioinequivalent products (i.e., to prevent a false positive result of bioequivalence) that a placebo group must be included. In addition to the test product being shown to be clinically equivalent to the reference product, both the test and reference product should be shown to be statistically superior to the placebo group.

In acne and rosacea studies, the percent reduction in inflamed lesion count at the end of treatment is often in the range of 50% to 60% and the corresponding decrease in the placebo group is in the 30% to 40% range. However, when the therapeutic response rate of the RLD is high and well-characterized and the placebo cure rate is known to be extremely low, the inclusion of a placebo arm in such study would appear to add nothing of scientific value to the study and should be carefully considered. To illustrate this point consider a clinical endpoint study with topical imiquimod 5% cream in the treatment of lesions of sBCC. In this case, an appropriate clinical endpoint could be the proportion of patients considered a clinical cure at the end of treatment. Clinical cure in this case would be defined as 100% eradication of baseline lesions. Based on many studies conducted with this product one would estimate approximately 80% of the patients in the reference group would be considered a clinical cure at the end of treatment. Placebo has minimal effect on sBCC lesions and the placebo cure rate would be expected to be 0% to 2% (8,28). Thus, the use of a placebo arm has no scientific value and is extremely difficult to justify.

Certainly the use of placebo treatment arms in studies where the population being treated is pediatric is even more problematic. Review of the SBOA for Ciclopirox/bethamethasone otic suspensions, which are indicated for the treatment of outer ear bacterial infections (a disease predominantly affecting the young) reveals the absence of any placebo-controlled trials as part of the NDA application. Consideration of a placebo arm in a similar bioequivalence study would certainly be unacceptable to most investigators and IRBs.

Patient Numbers

The number of patients required to participate/complete a clinical endpoint bioequivalence study is driven by five major factors:

1. the mean change from baseline or the anticipated clinical cure rate expected to be seen in the study with the reference product,
2. the mean change from baseline of the anticipated clinical cure rate expected to be seen in the study with the placebo group (if one is required),
3. the anticipated variance of the data set (the standard deviation),
4. the amount of confidence in the test formulation with regard to its comparability to the reference product, and
5. the proportion of enrolled subjects anticipated to be eligible for inclusion in the appropriate statistical analysis.

Three study populations are usually included in the statistical analysis. The analysis of bioequivalence for the primary endpoint is usually performed using the Per Patient Population (PPP). Although there is not a standardized definition of what constitutes the PPP, the following appears to be well accepted:

1. patients who met the inclusion/exclusion criteria,
2. patients who completed the final study visit within the protocol required window,
3. patients who were compliant with the dosing procedures of the study,
4. patients who did not use any restricted medications during the study, and
5. patients terminated from the study early for lack of efficacy should also be included in the PPP.

Analysis of superiority of the test and reference products to placebo or other comparator product and analysis of bioequivalence of secondary endpoints and superiority is performed using the Intent to Treat (ITT) population. The true definition of an ITT is all those patients in the PPP *plus* all those patients who were randomized and completed a second study visit after starting treatment. Sometimes, a modified ITT is used in this analysis which excludes patients who had severe protocol deviations such as not using the study medication as required or using prohibited medications between the baseline and second visit.

Analysis of safety must include all subjects who were randomized and used study drug on at least one occasion and this is generally referred to as the Safety Population.

It should be understood that it is more difficult to demonstrate the statistical bioequivalence of a generic product, than it is to demonstrate that the same product is superior to placebo. Therefore, the first priority is to ensure that the study is designed and powered appropriately to meet the primary objective in the PPP. After this has been completed then additional analysis can be performed to estimate the size of the placebo group in the ITT.

It is usually difficult to obtain information on the anticipated standard deviation of the expected change in mean difference. Often a standard deviation of 50% of the mean is used. However, this can lead to an under powering of the study design. For example in two recent rosacea studies the mean reduction in inflamed lesion count from baseline with the reference product was 9.8 and 9.7; yet the standard deviations were ± 9.0 and ± 10.2 , respectively. In the same studies the reduction in inflamed lesion count associated with the placebo was 7.14 ± 14.2 and 7.5 ± 10.6 . In both studies bioequivalence was met and demonstration of superiority achieved. Each study enrolled over 600 patients in a 1:1:1 ratio of test:reference:placebo.

From the preceding example it should be apparent that a successful bioequivalence study may depend upon an extremely large number of

patients. It is essential therefore that the study protocol be designed in such a way as to minimize the variability and to maximize the potential for success.

Figure 2 shows the subject numbers that would be needed in each treatment group to meet 90% CI for a dichotomous end point using the two, one-sided test procedure (29). In terms of sample size a “worst case” scenario would be an anticipated “cure rate” of exactly 0.5 (50%) for the reference group. In such a case, if the generic product exactly mimicked this cure rate then 117 subjects/treatment group would provide 80% statistical power against a false assumption of bioequivalence ($\alpha = 0.05$). As the “cure rate” increases or decrease away from 0.5 the number of subjects decreases. It is extremely unlikely that a generic product will provide exactly the same cure rate as the reference product. In practice, a small difference in cure rates, either positive or negative would not be considered to hold clinical significance. However, the effect of even a relatively small difference in “cure rates” can have a dramatic effect on the number of patients required. Even small differences between the products will require a significant increase in subject numbers, with a difference of ± 0.1 requiring an approximate tripling of the patient population.

In calculating subject numbers an allowance must be made for screen failures and drop out rates. In studies with microbiological endpoints such as *T. pedis* the number of patients who enter the study with clinical symptoms but who subsequently are found to have negative cultures at baseline can be as high as 50%. In other studies, especially those with long treatment durations, the drop out rate can often be as high as 30%.

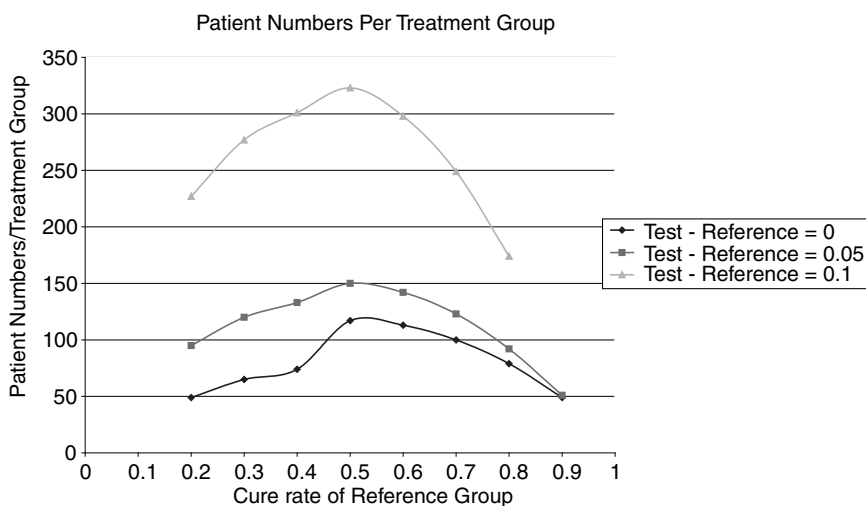


Figure 2 The number of patients required per treatment group for a dichotomous end point of “clinical cure” to demonstrate bioequivalence using the two, one-sided confidence interval approach with Yates correction factor (power = 80%; $\alpha = 0.05$).

An allowance for patients who drop out from the study must also be built into the subject number calculation.

In a study with a topical antifungal indicated for *T. pedis*, such as ciclopirox 0.77% cream, the anticipated “therapeutic cure” rate would be expected to be around 60% (a “therapeutic cure” is a patient who is both a “microbiological cure” and a “clinical cure” at the end of treatment). For a test product that had a “therapeutic cure” rate that differed by 5% from that of the reference product, 142 subjects in the PPP would be required in each treatment group. However, allowing for patients who have negative baseline mycology and for patients who drop out, 230 subjects in each of the active treatment groups and 115 in the placebo group should be considered (total number enrolled = 575 patients). This should ensure a PPP of 142 in the test and reference groups.

If superiority testing against a placebo is also a required part of the protocol design, then a calculation of the number of patients required in the placebo group must also be performed. Demonstrating statistical superiority never requires more patients than required to demonstrate bioequivalence. Additionally, in many cases the placebo “cure rate” is less than half that anticipated in the active groups, and therefore, less subjects can be included in the placebo group. For example a 2:2:1 or even a 3:3:1 ratio of test:reference:placebo may suffice. Where the active treatment cure rate is low and/or the placebo cure rate is greater than half that seen in the reference group then an equal number of subjects in the placebo group should be considered (1:1:1).

Inclusion/Exclusion Criteria Considerations

The selection of patients for bioequivalence endpoint studies is a difficult balance of attempting to ensure that the maximum change from baseline can be achieved, that variability is minimized, and that operationally a large enough patient base can be recruited in a reasonable time frame.

In addition, a review of other available treatment options in the country/location that the study is intended to be conducted is an important part of this review. For example the increasing use of over the counter antacids and H₂ antagonists has meant that many patients with gastric and duodenal ulcer now self-medicate to treat these diseases. Standard treatment protocols in the United States also recommend the use of antibiotics if the patient has also been shown to have *H. pylori* infection. Thus, it is increasingly difficult to find treatment-naïve patients or patients who are washed out from these medications to conduct large studies.

Baseline Stability and Comparability

As all clinical equivalence studies rely on evaluating some change from baseline, it is important that the possible differences between treatment

groups be minimized. Additionally, when considering inclusion/exclusion criteria the entry criteria should be carefully considered such that it is anticipated a measurable change from baseline can be identified. In the case of a placebo controlled study a change that will differentiate the test and reference groups from baseline must also be considered.

The usual primary endpoint in acne studies is change in inflamed lesion count from baseline. Most prescription topical acne products are indicated for mild to moderate acne, with very mild acne usually being treated with good facial hygiene and more severe acne with oral products. Although there is no universally accepted definition of mild to moderate acne based on actual inflamed lesion count, a patient with a range of 10 to 80 inflamed lesions would probably be considered to fall within this category. This is a very wide baseline window, and even with large patient numbers could potentially lead to imbalanced treatment groups. Additionally, lesion counts will fluctuate during a study, based on a large number of external and physiological factors that are usually not under the investigators control. To minimize the baseline variability, consideration for narrowing the definition of inclusion should be considered, without excluding patients who would be indicated for using the drug or for making recruiting overly onerous. Setting a minimum baseline count of 20 inflamed lesions would certainly be useful in such a case, to help ensure the test and reference product would at the end of treatment, be able to be statistically separated from the placebo group.

The requirement for the use of a comparative placebo group in an actual study is discussed in section “Use of Placebo Treatment Arm.” For studies of AR, a blinded one-week placebo lead in period is often used to exclude subjects whose AR may be transient or variable. In such studies, the placebo lead in exclusion rate can be 30% or higher. Such a protocol should be written so that sufficient patients are enrolled into the placebo lead in period to ensure that enough qualify to be randomized. In AR studies when it is desirable to randomize 600 patients, 1000 patients may need to enter the placebo lead in period.

Reducing Variability

Many clinical endpoint studies with topical products depend on an individual, sometimes the patient, to make assessments of the severity of a disease state and it is these assessments that form the basis of the data set, upon which bioequivalence is going to be determined.

For a 600 patient study in psoriasis, 30 or more investigative sites may be used to ensure that patient enrollment timelines are kept within a reasonable timeframe. In an ideal situation, each clinical site would have one investigator who was responsible for making all the patient assessments for that location. In reality, however, several investigators will be making the

assessments depending on patient and investigator availability. In such a study it would not be unusual to have 60 or 70 different people making assessments and thus providing input into the data set.

To minimize the variability in the data set, it is crucial that a well designed training and validation program be executed before the study commences. Only those investigators who have been able to demonstrate that they are able to make assessments that are consistent with a known standard, should participate in the study. The use of unambiguous and well-defined grading scales is particularly important. An example of such a rating scale for “plaque elevation,” one of the signs used in the assessment of psoriasis is provided in Table 2.

The use of well-defined scales is even more important when patients are required to assess their own symptoms. These scales should be simple and based on symptoms that the patient is easily able to relate to and quantify. An example of rating scales for a patient’s self assessment of nasal irritation that could be used in an AR study is shown in Table 3.

Study Drug Blinding

To eliminate investigator and/or patient bias the study drugs must be appropriately blinded. Although the test drug/formulation is usually easy to blind, ensuring that the packaging is blinded can often be problematic. The use of an outer opaque shrink wrap covering on tubes of creams and gels will certainly cover the labeling of the reference product, although a different tube type or cap may be impossible to blind. For nasal spray products, the use of an outer masking device that does not affect the performance of characteristics of the delivery device can also be used.

Packaging the products in identical boxes will also help prevent the clinical staff from being able to identify the drug product. The use of an independent dispenser/receiver at each location, somebody who is not involved in any of the efficacy assessments, will further ensure that the study

Table 2 An Example of a PGA Rating Scale that May Be Used in the Assessment of Plaque Elevation for Psoriais

0 = Clear	No evidence of plaques above normal skin level
1 = Almost clear	Slight, just discernable elevation above normal skin level
2 = Mild	Discernable elevation above normal skin level upon examination, but not pronounced
3 = Moderate	Definite plaque formation with rounded/sloped edges to plaque
4 = Severe	Marked elevation with hard, distinct edges to plaque
5 = Very severe	Very marked elevation, very hard and sharp edges to plaque

Abbreviation: PGA, physician’s global assessment.

Table 3 A Patient’s Self Assessment Rating Scale that May Be Used to Generate a TNSS in an AR Study

Patients will rank each of the following four symptoms on a scale from 0 to 3.
Nasal Congestion
Runny Nose
Sneezing
Itchy Nose
0= No symptom
1= Mild symptoms (Sign/symptom present, minimal awareness, easily tolerated)
2= Moderate symptom (definite awareness of sign/symptom, bothersome but tolerable)
3= Severe symptom (sign/symptom hard to tolerate, causes interference with daily activities and/or sleeping)

Abbreviations: TNSS, total nasal symptom score; AR, allergic rhinitis.

remains blinded. Individual sealed envelopes for each patient that accompany the study drug can be used to break the blind for a specific patient in the case of a medical emergency, ensuring that the complete study blind is not broken.

For studies intended for submission to the FDA, at the conclusion of the study and after the data has been analyzed, a copy of the randomization should be sent to each clinical site in a sealed envelope, in case of an FDA inspection which requires collection of drug retention samples (30).

DATA AND STATISTICAL ANALYSIS

It is beyond the scope of this chapter to provide a detailed description of the statistical methods that may be utilized in clinical endpoint studies. Development of a statistical plan that clearly identifies the primary and secondary endpoints, the populations that will be used in each analysis (e.g., PPP and ITT), and the specific tests that will be used, prior to the start of the study is critical. The protocol should also be used to clearly state a priori what result will be considered as having determined bioequivalence.

Prior to starting any analysis of the efficacy data, the treatment groups should be tested for comparability using methodology appropriate for the baseline variable (e.g., Scheffe’s paired comparison test for continuous variables such as age or lesion count or Cochran-Mantel-Haenszel for categorical parameters like gender and race). If a statistically significant imbalance is seen between groups then baseline adjustments may be needed prior to further analysis.

For multiple site studies, analysis of site by treatment interaction analysis should also be considered for the primary endpoints. Sites that have

enrolled very few patients in the study (<5%) and would thus not be expected to have any significant impact on the overall analysis may be combined for this analysis.

For dichotomous endpoints such as “clinical cure” or “mycological cure” the 90% CI of the proportional difference between the test and reference product should fall with the range -0.20 to $+0.20$.

The null hypothesis in such a test is:

$$H_0: P_T - P_R \leq 0.20 \text{ or } P_T - P_R \geq 0.20$$

The alternative hypothesis is:

$$H_A: 0.20 < P_T - P_R < 0.20,$$

where P_T = Proportion of patients in the test group considered a “cure” and P_R = proportion of patients in the reference group considered a “cure”.

The lower (L) and upper (U) 90% CI can be calculated (usually using Yates continuity correction REF) using the following formula:

$$L = (P_T - P_R) - 1.645SE - (1/n_T + 1/n_R)/2$$

$$U = (P_T - P_R) + 1.645SE - (1/n_T + 1/n_R)/2$$

where n_T and n_R are the number of patients in test and reference group, respectively. The standard error (SE) may be calculated as follows:

$$SE = (P_T(1 - P_T)/n_T + P_R(1 - P_R)/n_R)^{1/2}$$

If both $L \geq 0.20$ and $U \leq 0.20$, then the null hypothesis may be rejected and equivalence is concluded.

Handling continuous variables such as mean change from baseline in inflamed lesion count in acne or rosacea studies, or reduction from baseline in TNSS in AR studies is more challenging. The overall requirement to demonstrate bioequivalence is similar. In this case, the 90% CI of the ratio of the mean change from baseline in the test group compared to the mean change from baseline line in the reference group need to be within $\pm 20\%$ (90% CI $L \geq 80$ and ≤ 125). While parametric testing such as analysis of variance (ANOVA) based on the General Linear Model is preferred, the data is often not normally distributed. Log transformation or similar is usually not possible, as you are dealing with both positive and negative changes from baseline. In such cases nonparametric testing such as Wilcoxon Rank Sum Test may be considered.

In studies where a placebo group is included, and superiority testing is required, then standard statistical testing appropriate for the type of data set can be employed (Chi-square for dichotomous data and ANOVA for continuous variables are most commonly used). For skewed data sets non-parametric testing may be more appropriate.

CONCLUSION

Developing generic drugs using comparative clinical end point studies is clearly a more complicated process than developing oral dosage forms intended for systemic absorption. With the cost of clinical endpoint studies often running into several millions of dollars, the cost of entry into this market can be prohibitive to smaller companies. The rewards for those prepared to take risk, however, can be significant. The annual sales of Flonase® (fluticasone propionate) nasal spray in 2005 were estimated to be over \$1 billion.

With such large revenues at stake, it should also be recognized that the innovator company will also try and defend its market share by trying to exclude generics for as long a possible. The number of Citizens Petitions filed in recent years in the United States by companies trying to restrict the entry of generics into the market is a clear indication of this tactic. Regulatory bodies are therefore under increasing pressure in such to ensure that they are fully comfortable that the design and results of clinical end-point studies are defensible for approved generics.

The design, analysis and use of clinical endpoint studies to determine bioequivalence, continues to be one of much discussion and debate (31). It is hoped that this short introduction to this area of clinical research will be used to stimulate interest and open communication throughout all those involved, such that the medical community, and most importantly patients, can be assured of the efficacy and safety of marketed generic locally acting products.

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Evaluation of Bioequivalence of Highly Variable Drugs

Laszlo Endrenyi

Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada

Laszlo Tothfalusi

Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary

INTRODUCTION

The Problem of Assessing the Bioequivalence of Highly-Variable Drugs

Qualitative Exposition of the Problem

The assessment of bioequivalence (BE) of highly-variable (HV) drugs has been of great concern over the past several years. The issue was repeatedly a principal topic at several conferences (1–3) and also at an American Association of Pharmaceutical Scientists (AAPS)/Food and Drug Administration (FDA) workshop (4) and at a recent AAPS symposium (5). Regulatory agencies have also been considering the problem, especially in recent years.

There has been general agreement that HV drugs are characterized by a within-subject variation of at least 30% (1,4). With such large variations, the evaluation of BE becomes difficult unless the sample size used in a study is also large. The variation can be determined, in two-period BE studies, from the residual variation of an analysis of variance and, in multi-period BE investigations, from the directly estimated intraindividual variance.

To understand this statement, recall that in BE studies, the geometric means of the kinetic responses [e.g., of areas under the concentration vs. time curves (AUC) and of maximum concentrations ($C_{\max}$)] of two drug

products are typically compared. BE is generally declared if the 90% confidence interval (CI) around the ratio of the two geometric means (GMR) is within preset regulatory limits. Regulators most often require that the BE limits for the GMR be between 0.80 and 1.25.

The CI is proportional to the estimated variation. Consequently, with rising variations, it is increasingly likely that the recorded CI would reach beyond the BE limits.

The (CI) is also reciprocally proportional to the square root of the sample size. Therefore, with increasing variability, it is possible to keep the interval within the BE limits by substantially increasing the number of subjects in a study. As will be noted below, the required sample size can rapidly grow well beyond the typical 24 to 36 subjects required in a BE investigation. Such a possible need for large samples can raise practical and ethical issues.

Quantitative Exposition of the Problem

The usually applied criterion for the determination of BE involves the application of the two one-sided tests procedure (6). Accordingly, the average logarithmic kinetic responses of the test and reference formulations (μ_T and μ_R , respectively) are contrasted. The acceptance of BE is declared if the difference between the logarithmic means is between preset regulatory limits. The limits (θ_A) are generally symmetrical on the logarithmic scale, and usually equal $\ln(1.25)$. Consequently, the criterion for the determination of average bioequivalence (ABE) is:

$$-\theta_A \leq \mu_T - \mu_R \leq \theta_A \quad (1)$$

In a BE study, the individual kinetic responses are evaluated from the measured drug concentrations. The means of the logarithmic responses of the two formulations (m_T and m_R) are calculated; these sample averages estimate the true population means (μ_T and μ_R). In addition, a variance (s^2) which is a measure of the within-subject variance (but not always identical with it) is estimated for each kinetic response.

Following a typical *two-period, balanced BE study*, the ABE criterion expects that the difference between the estimated logarithmic means ($m_T - m_R$) be, together with its (usually 90%) CI, within the regulatory limits:

$$-\theta_A \leq (m_T - m_R) \pm t(2s_{\text{res}}^2/N)^{1/2} \leq \theta_A \quad (2)$$

The last term calculates the confidence limits. N is the number of subjects, t is the student's statistic at the probability of $\alpha = 0.05$ with $N - 2$ degrees of freedom, and s_{Res}^2 is calculated as the residual mean square in the relevant analysis of variance. It contains and reflects the within-subject variance but, in this analysis, is not identical with it.

As the estimated variation rises, it becomes more difficult to remain within the regulatory BE limits and it is less likely that the two drug products

can be claimed as being bioequivalent. In order to maintain the probability of accepting BE, it would be necessary to maintain the magnitude of the term $(2s_{\text{Res}}^2/N)^{1/2}$ in Equation (2), and therefore increase the sample size.

Implications of the Usual Regulatory Conditions Applied to HV Drugs

The approach based on the two one-sided tests procedure, and therefore on Equation (2) has served the regulatory evaluation of BE for many drugs very well, particularly when there were no kinetic or investigational complications and the within-subject variation was not high.

However, the possible need to use large numbers of subjects for the assessment of HV drugs raises practical and ethical issues. Benet (7) questioned the appropriateness of exposing large numbers of volunteers to drugs even if these could be deemed to be generally safe. He noted that “for drugs exhibiting high pharmacokinetic variability, little clinical relevance results from requiring a very large number of subjects to approve a standardized CI which probably has no safety and efficacy implications” and for highly variable drugs with wide therapeutic indices it should not be necessary to study an excessive number of subjects to prove that two equivalent products meet preset (one size fits all) statistical criteria (8).

The large sample sizes required can have awkward consequences such as an increase in the cost of such an investigation. Even if this consideration in itself is not considered important, the consequences could be serious. First, generic drug products or more advanced formulations of new drugs may simply not be developed, a fact to be regretted by the consumers, the patients. Second, the failure rate of studies may rise as the (mainly) within-subject variation increases. This has been the observation of a large contract research organization (9) and is widespread anecdotal information emanating from several similar organizations. Third, the development of these drug products could be strongly distorted when smaller generic pharmaceutical companies will be unable to compete with larger ones.

Perhaps even more important, it may not be ethical to expose unusually large numbers of healthy volunteers to a drug even if it is considered to be generally safe, just for the sake of satisfying a traditional statistical criterion.

A feature of the difficulties involving the determination of the BE of HV drugs is that, under typical conditions, a drug product may even not be found to be bioequivalent with itself. This has been demonstrated with verapamil formulations (10) and, in three parallel studies, on three chlorpromazine products (11).

Issues involving the determination of BE are also relevant to the characterization of new formulations during drug development. Thus, the practical and ethical questions considered for HV drugs are particularly important also during the formulation and development of such drug products when the safety and effectiveness have not yet been fully demonstrated.

PROPOSED SOLUTIONS TO RESOLVE THE PROBLEM OF THE ASSESSMENT OF THE BE OF HV DRUGS

Relax a Regulatory Requirement

In Canada, the regulatory authority generally requires that only the point estimate of the GMR for $C_{\max}$, but not its 90% CI, be between the regulatory limits of 0.80 to 1.25 (12). This relaxed requirement applies generally and does not aim specifically at HV drugs. Nevertheless, it enables the satisfactory determination of BE of several HV drugs since the variation of $C_{\max}$ is usually higher than that of AUC and, therefore, the determination of BE can often fail because of the wide CI of $C_{\max}$.

$C_{\max}$ relies on a single observation and, therefore, usually has higher variation than AUC. Consequently, when the 90% CIs of GMR for both AUC and $C_{\max}$ have to satisfy the regulatory criterion then the determination of BE can often fail because of the unrealistic wide CI of $C_{\max}$. The Canadian regulatory requirement for $C_{\max}$ clearly takes such an unnecessarily stringent constraint into account by considering the point estimate only.

Stepwise Widening of BE Limits

Taking into consideration that $C_{\max}$ often exhibits larger variation than AUC, it has been suggested that the BE limits of its GMR could be widened from 0.80 to 1.25 to 0.75 to 1.33 or even to 0.70 to 1.42. Notably, while the Committee for Proprietary Medicinal Products of EMEA (CPMP) Guidance in Europe expects the usual BE limits of 0.80 to 1.25 for both $C_{\max}$ and AUC, it allows that: "In certain cases, a wider interval may be acceptable. The interval must be prospectively defined (e.g., 0.75–1.33) and justified, addressing in particular any safety and efficacy concerns for patients switched between formulations" (13).

A difficulty with the stepwise widening is the discontinuity of the regulatory expectations. For instance, if BE regulatory limits of 0.75 to 1.33 were adopted then an observed 90% CI of, say, 0.82 to 1.32 would be accepted to demonstrate BE whereas a 0.80 to 1.34 interval would be rejected. Moreover, the broader limits offer only a partial remedy to the issue at hand since the problem still arises when the within-subject variation is very high. For instance, the regulatory range of 0.75 to 1.33 could be satisfactory with within-subject variations of, say, 35% but would be of little help with variations of, say, 60% (9).

Steady-State BE Studies (Multiple Dosing)

It has been demonstrated that observed variations of the BE metrics (AUC and $C_{\max}$) are often lowered after multiple dosing. Blume et al. (14,15) showed this by comparing measures obtained at steady state with those

from single-dosing studies of HV drugs such as loratadine, verapamil and propafenone.

Some of the theoretical investigations and simulations substantiated these results and provided a background where it was seen that the variation of $C_{\max}$ was lower at steady state than after single dosing when drug accumulation was high (16) and when the variation of the absorption rate constant was substantially higher than that of clearance (17,18). However, it was argued that the reduced variation of $C_{\max}$ at steady state was due to its diminished kinetic sensitivity to the rate of absorption (19,20).

However, under other conditions, the BE metrics obtained at steady state were not found to be advantageous. For example, $C_{\max}$ actually exhibited higher variation at steady state than following a single drug administration if the variations of clearance and distribution volume were higher than that of the absorption rate (17,18). As a result, El-Tahtawy et al. (21) concluded, “multiple-dose designs may not be the best solution for assessing BE of highly variable drugs.” Moreover, measurements of $C_{\max}$ have an upward bias, particularly in the steady state, and especially with high drug accumulation (22). This provides further affirmation for the conclusion of El-Tahtawy et al. (21).

Switchability and Individual BE

For several years, serious consideration was given to the model of individual BE. The reason was that it was based on the important concept of switchability between drug formulations (23–25).

The principle of the model states that in order to evaluate the BE of a generic product, regulators should compare the switchability from a reference (R) to a test (T) product, within individuals, with repeated observation within subjects. Consequently, the (T-R) difference of a kinetic characteristic such as AUC or $C_{\max}$, should be compared with an (R-R) difference. Therefore, the ratio (T-R)/(R-R) should be determined.

The model developed from this principle expanded the simple contrast used for ABE (Equation (1)). Not only the (squared logarithmic) means of the two formulations are compared, $(\mu_T - \mu_R)^2$, but also their within-subject variances, $(\sigma_{WT}^2 - \sigma_{WR}^2)$. In addition, the variance component of the so-called subject-by-formulation (σ_D^2) is estimated. Consequently, the numerator of the regulatory model for individual BE contains three terms (24–28):

$$[(\mu_T - \mu_R)^2 + (\sigma_{WT}^2 - \sigma_{WR}^2) + \sigma_D^2] / \sigma^2 \leq \theta_1 \quad (3)$$

θ_1 is the regulatory criterion for individual BE. Since the numerator in Equation (3) contains three terms instead of one in Equation (1), it was suggested that the criterion θ_1 be larger than the θ_A established for ABE (25,27,28).

The denominator in Equation (3) is a variance. It is constant when the within-subject variance is not higher than a predetermined value (σ_0^2), i.e., when $\sigma_{WR}^2 \leq \sigma_0^2$. Alternatively, the variance in the denominator equals the intra-individual variance of the reference formulation ($\sigma^2 = \sigma_{WR}^2$) when the within-subject variance is higher than the predetermined value ($\sigma_{WR}^2 > \sigma_0^2$), i.e., in the case of HV drugs (25,27,28). Consequently, this so-called *mixed strategy* applies scaling, which is referred to as either “constant scaling” at low variations when $\sigma_{WR}^2 \leq \sigma_0^2$ or “reference scaling” at high within-subject variance when $\sigma_{WR}^2 > \sigma_0^2$.

Expanding the BE Limits

Boddy et al. (29) recommended that, for HV drugs, the distance between the regulatory limits should be widened in proportion to a standard deviation that is a measure of the within-subject variation (σ_W). The basis of their arguments was that the objective of regulation is to demonstrate BE with a *reasonable* number of volunteers. Boddy et al. showed that, with their approach, BE could be shown with a fixed number of volunteers at any within-subject variability. The power depends only on the assumed difference between the means and on the number of volunteers but not on the within-subject standard deviation.

In particular, Boddy et al. (29) proposed the following regulatory criterion:

$$-k\sigma_W \leq \mu_T - \mu_R \leq k\sigma_W \quad (4)$$

The constant, k , should be set according to an assumed, reasonable number of volunteers. Boddy et al. recommended, for two-period BE studies, that $k = 1.0$.

Scaled Average Bioequivalence

It has been suggested (30,31) that, for HV drugs, the usual regulatory criterion of ABE [Eq. (1)] could be scaled by a standard deviation:

$$-\theta_S \leq (\mu_T - \mu_R)/\sigma_W \leq \theta_S \quad (5)$$

Here the standard deviation applied for scaling (σ_W) is again related to the within-subject standard deviation. In two-period studies, it is the residual standard deviation (σ_{Res}) that is estimated from the relevant analysis of variance. In replicate-design investigations, σ_W is generally the within-subject standard deviation of the reference formulation (σ_{WR}). Thus, the scaling factor of scaled average bioequivalence (SABE) in Equation (5) has similar features to the scaling factor σ of individual BE in Equation (3).

$\pm\theta_S$ are the regulatory BE limits for SABE and their suggested magnitudes will be discussed later.

It has been demonstrated that, for HV drugs, the SABE approach requires fewer subjects than unscaled ABE (31). Calculations involving SABE and features of the method are described in the next section.

Recent Considerations by Regulatory Agencies

In recent years, the problem of determining BE of HV drugs has gained the attention of regulatory agencies. FDA in the United States considered it for some years in the context of individual and population BE. (27,28,30,32) More recently, its attention has turned towards exploring the issue by the customary approach of ABE. FDA has established a Working Group on the BE of Highly Variable Drugs and Drug Products which intends to develop a guidance dealing with the problem. The Group made presentations to a meeting of its Advisory Committee in 1994 (33) and at an AAPS symposium in 2005 (5).

Other regulatory agencies have also considered the issue of bioequivalence assessment of HV drugs. The Therapeutic Products Directory (TPD) of Health Canada did not think that HV drugs would require special consideration (12). However, as noted earlier, the Canadian agency requires only that the point estimate of the $C_{\max}$ ratio but not its confidence limits be between the regulatory limits of 0.80 to 1.25. Since $C_{\max}$ is usually more variable than AUC, the problem of BE for HV drugs is less apparent in Canada.

The South African Medicines Control Council recommends the use of SABE and/or expanded BE limits for $C_{\max}$ (34,35). The European Agency for the Evaluation of Medical Products (EMEA) has begun to give serious consideration to the problem (36).

ANALYSIS OF BE BY SABE

Background and Procedures

Evaluation of BE with a scaled equivalence test, including by SABE, has been suggested by several leading statisticians (24,37–39). Wellek, in his monograph (40), gave a very thorough and mathematically rigorous treatment of the topic. The concept of SABE will be presented in a pragmatic fashion. Current controversial and still open questions will also be discussed; they require consideration also by the various regulatory authorities.

Regulatory guidelines (13,20) advise that replicate designs with three or more periods be used in investigations for evaluating the BE of HV drugs and drug products, and the evaluation of the resulting data can be challenging. Therefore, calculations will also be considered for performing a BE test for HV drugs. Chow and Liu (41) provide a general review for the evaluation of BE studies and Patterson and Jones (42) have presented a more recent, highly readable reference of the topic.

As seen earlier, the decision criterion for ABE is:

$$-\theta_A \leq \mu_T - \mu_R \leq \theta_A, \quad (1)$$

while the criterion for SABE is:

$$-\theta_S \leq (\mu_T - \mu_R)/\sigma_W \leq \theta_S. \quad (5)$$

The formula for SABE is deceptively simple and hides the fact that there are several questions that should be answered so that SABE can be used in practice. Some of the questions have definite and straightforward answers while others do not. These questions will be considered and the most important will be addressed.

Application of SABE in Studies of HV Drugs

Mixed Regulatory Strategy for HV Drugs

A mixed strategy for the application of individual BE was described earlier. By analogy, it was suggested that a similar mixed model could be utilized for evaluating the ABE of formulations of HV drugs (30,31,40,43). Again, a so-called switching variation (σ_0) separates the region of HV drugs from the range of drugs having low variability. At low variations ($\sigma_W \leq \sigma_0$), i.e. below and until reaching the switching variation, unscaled ABE will be calculated as at present. At high variations ($\sigma_W > \sigma_0$), a procedure that is relevant to the analysis HV drugs, such as SABE, is applied.

The mixed strategy is illustrated in [Figure 1](#). It depicts the regions of low and high variation and their BE regulatory limits.

Study Design

Replicate designs have important merits in determinations of BE for HV drugs products (28,31,42,44,45). First, they require fewer subjects than two-period studies for attaining the same statistical power. As a rule of thumb, about half as many volunteers are needed to conduct a four-period study than a two-period investigation. However, the number of subjects required in a replicate design study could increase somewhat which could be due, in part, to the longer duration of such investigations resulting in dropouts. Replicate design studies yield additional, important information such as permitting the within-subject variances of both drug products to be estimated. This enables their comparison, such that if the products are indeed dissimilar, then, possibly only one of its formulations could be highly variable (*vide infra*) as opposed to the drug itself. The estimated intrasubject variance of the reference product can thus also serve as the standardizing term in applications of SABE.

Replicate designs can also reveal and estimate any subject-by-formulation interaction and the corresponding variance component. This

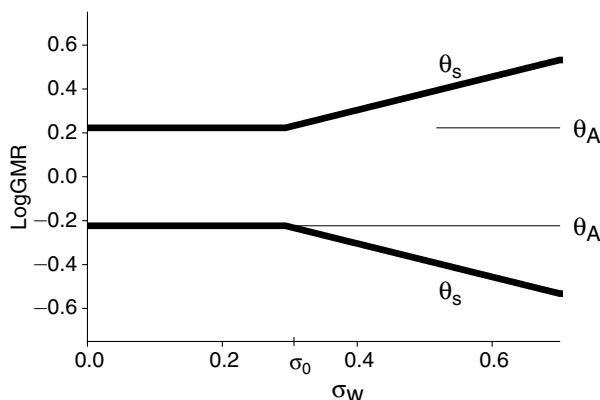


Figure 1 Mixed strategy for the determination of average bioequivalence. When the variation (σ_W) of a drug that is a measure of the within-subject variability, is below a “switching variation” (σ_0) then the regulatory limits are the customary $\pm \ln(1.25)$ which are used for the evaluation of ABE. However, in the range of HV drugs when σ_W exceeds σ_0 , the apparent regulatory limits increase proportionately with σ_W . In principle, it is better to use the approach of SABE in the region of HV drugs. *Abbreviations:* BE, bioequivalence; ABE, unscaled average bioequivalence; SABE, scaled average bioequivalence; HV, highly-variable.

component was thought, a few years ago, to be of great potential importance in assessments of BE and in explorations of drug behavior (27,32). However, it is highly unlikely that a single BE study would have the statistical power to detect a significant interaction (46) whereas it is possible that combinations of several investigations could identify meaningful interactions.

Two-period studies have the advantage of relative simplicity. A statistical procedure has been developed which still permits the estimation of the ratio of the within-subject variances between the two formulations (47–49). However, the characteristics of these procedures still need to be evaluated.

Under some circumstances, replicate designs cannot, in practice, be applied and two-period BE studies have to be conducted. The conditions include investigations involving drugs with long half-lives (50,51) as well as many biotechnological preparations and those used in veterinary medicine (personal communication). Procedures for these conditions will have to be explored.

Setting the BE Limits

There are currently no clear regulatory guidances on how to set the regulatory BE limits for HV drugs, i.e. what should be the value of θ_s when

SABE is used? Tothfalusi and Endrenyi (43), however, noted that this is a straightforward issue if the mixed strategy is followed and the principle of continuity is accepted. Continuity requires that when the estimated variation (s_w) equals the switching variation (σ_0) then it should not matter which method, SABE or ABE, is applied since both approaches should yield the same result. As already noted, there is a general understanding (1,4) that drugs are considered to be highly variable if their intra-subject coefficient of variation is higher than 30 %. Using the conversion formula of

$$\sigma^2 = \ln(CV^2 + 1) \quad (6)$$

to calculate the variance on the logarithmic scale, $\sigma_0 = 0.2936$ is obtained for the switching variation. Therefore, in order to maintain continuity, the regulatory limits for SABE should be $\pm \log(1.25)/0.2936 = \pm 0.760$ (43). Interestingly, Wellek (40) arrived at practically the same conclusion from a completely different direction. He defined the equivalence limits in terms of quantiles, and showed that the usual 20% confidence limit requirement corresponds to setting the SABE limits to ± 0.74 .

Interpretations of SABE

Tothfalusi and Endrenyi (43) listed several interpretations of SABE. Conceptually SABE is not new, it has appeared in the literature sometimes in disguised forms and as a solution of surprisingly differing problems.

SABE and the Therapeutic Switchability Model

The principle of switchability among drug formulations within subjects, has been described earlier. A consequence of the principle was the regulatory model for individual BE (Eq. (3)). When the two contrasted drug products have the same within-subject variance ($\sigma_{WT}^2 = \sigma_{WR}^2$) and the variance component for the subject-by-formulation is zero ($\sigma_D^2 = 0$) then, for HV drugs, the model for individual BE [Eq. (3)] reduces to that for SABE [Eq. (5)] (30,39,43,52).

Consequently, SABE can be interpreted within the framework of therapeutic switchability.

SABE as a Correct Solution to the Problem of Expanding BE Limits

The proposal (29) to expand the BE limits in proportion to σ_w , the within-subject standard deviation, was discussed earlier. Tothfalusi and Endrenyi (43) argued that the two one-sided testing procedure of Schuirmann (6) cannot be applied in this case, because the BE limits themselves become random variables. Dividing all sides of Eq. (4) by σ_w results in

$$k \leq (\mu_T - \mu_R)/\sigma_w \leq k, \quad (7)$$

which, according to Equation (5), is the SABE problem. Indeed, as we will show later, this method and SABE have a number of common features. Consequently, the goal set up Boddy et al. (29) can be achieved by SABE.

SABE and Distance Metrics

This is a purely mathematical concept, in a very elegant mathematical theory developed by Dragalin et al. (53). They showed that in the function space of probability distributions, the scaled difference $[(\mu_T - \mu_R)/\sigma_w]$ is a distance metric if two probability density functions (corresponding to the two formulations) have the same variance. The metric concept can be further generalized by dropping the assumption of identical variances; in this case a formula similar to but not identical with that of individual BE [Equation (3)] is obtained.

SABE as an Equivalence Test for Effect Sizes

Quantities such as $(\mu_T - \mu_R)/\sigma_w$ are widely used in diverse areas of science including psychology (54), educational research (55), quality control (56), and medicine (57). The concept was originally introduced by Cohen (58) under the term of *standard* (or *standardized*) *effect size*. It is used to measure a scale invariant difference between two groups. Arguments have been presented that an index like the effect size should measure the clinical significance of medical treatments (59). In this framework, SABE could be interpreted as a test assessing whether a standard effect size is smaller than a predefined threshold of clinical significance.

Computational Procedures for SABE

Calculation of the Upper Confidence Limit

Similar to unscaled ABE, there are generally two approaches to perform an SABE test. The first is the two one-sided tests procedure of Schuirmann (6). Wellek (40) gives an example how to modify this test to evaluate SABE from data of 2×2 BE trials. An alternative test for the same problem has been developed by Tothfalusi and Endrenyi (43). This test is based upon the classical CI approach and uses the non-central T distribution to calculate the confidence limits. A disadvantage of both methods is that it is not known at present how they can be extended when the common within-subject variation ($\sigma_{WT} = \sigma_{WR}$) cannot be taken for granted. In this case, according to the switchability model (23–25) [see earlier, Equation (3)], the correct scaling term is the within-subject standard deviation of the reference drug product (σ_{WR}).

A general procedure for calculating the confidence limit with SABE which can be used in any of these settings, has been given by Tothfalusi et al.

(31) and is based on the method of Hyslop et al. (60). The calculations are summarized below where the starting point is the regulatory model for SABE:

$$-\theta_S \leq (\mu_T - \mu_R) / \sigma_W \leq \theta_S \quad (5)$$

For computational purposes, the squared form of Equation (5) is used:

$$(\mu_T - \mu_R)^2 / \sigma_W^2 \leq \theta_S^2. \quad (8)$$

This expression can be rearranged into a linear form:

$$(\mu_T - \mu_R)^2 - \theta_S^2 \cdot \sigma_W^2 \leq 0 \quad (9)$$

The two independent terms can be estimated, respectively, by:

$$E_m = (m_T - m_R)^2 \text{ and} \quad (10)$$

$$E_s = \theta_S^2 \cdot s_W^2$$

The confidence limits for the two terms in the rearranged BE criterion are:

$$C_m = [\text{Abs}(m_T - m_R) + t_{\alpha, N-S} \text{SE}]^2 \quad (11)$$

$$C_s = \theta_S^2 \cdot (N - S) \cdot s_W^2 / \chi_{\alpha, N-S}^2$$

Here, t and χ^2 are inverse cumulative distribution functions evaluated at the probability level of $\alpha=0.95$ and with $N - S$ (where N is the number of subjects and S the number of sequences) degrees of freedom. SE is the standard error of the difference between the means.

The squared lengths of the CIs are:

$$L_m = (C_m - E_m)^2 \quad (12)$$

$$L_s = (C_s - E_s)^2$$

Finally, the confidence limit (CL) for the rearranged BE criterion (Eq. (9)) is:

$$\text{CL} = E_m - E_s + (L_m + L_s)^{1/2} \quad (13)$$

Scaled ABE is rejected if the calculated upper 95% confidence limit is positive, and accepted (“not rejected”) if the upper 95% confidence limit is zero or negative. The described algorithm is a numerical approximation method; the approximation may not be good if the degrees of freedom are low (<12). However, our limited simulations show that the approximate procedure gives very close results to the theoretically correct methods.

A Numerical Example

The following short numerical example illustrates the calculation of the upper confidence limit. The results from a hypothetical four-period, two-sequence BE study with 24 subjects are:

$$m_T - m_R = 0.050$$

$$SE(m_T - m_R) = 0.0884$$

$$s_W = 0.45$$

Then $C_m = 0.0407$, $C_s = 0.0759$, $E_m = 0.0025$ and $E_s = 0.1170$. The upper confidence limit (CL) of the linearized criterion is -0.0584 . CL is negative and, therefore, SABE has been demonstrated.

Statistical Properties of SABE

Statistical Power and Producer Risk

The statistical properties of SABE were studied by simulating BE studies and then contrasting the power curves (Figs. 2–5) of ABE and SABE. The power curves depict, under various conditions, the proportion of simulated studies in which BE is successfully declared. The proportion is evaluated as the true GMR is raised gradually from $GMR = 1.0$ towards higher values. The highest proportion is observed when the two formulations are in fact bioequivalent (i.e., when $GMR = 1.0$). The complement of this proportion (i.e., 1.0 –the proportion) is the producer risk which is the probability of recording the absence of BE when the two drug products are actually bioequivalent.

A computer program, was written by the authors in Fortran (Compaq Visual Fortran, ver. 6.1) to simulate, under each condition, ten-thousand BE trials with two-, three- and four-period designs, and with different $\mu_T - \mu_R$ values (i.e., with different GMR's), numbers of volunteers, and intra-subject variations. In each case, it was recorded whether the trial was or was not successful and the results are presented in Figures 2 through 5. The criterion for BE decision was based on calculating the upper confidence limit [Equation (13)] and utilizing the proposed BE limit of $\theta_s = 0.760$ and switching variation of $\sigma_0 = 0.294$.

Figure 2 shows one the most important features of SABE, namely that the producer risk [i.e., the complement of the Acceptance% or $(100 - \text{Acceptance } \%)$ at $GMR = 1.0$] is independent at about 40% of the intra-subject variation. Also, SABE has substantially higher power than ABE. The power curve at the intra-subject $CV = 30\%$ is slightly different from the others. This is reasonable because the mixed strategy was used to evaluate

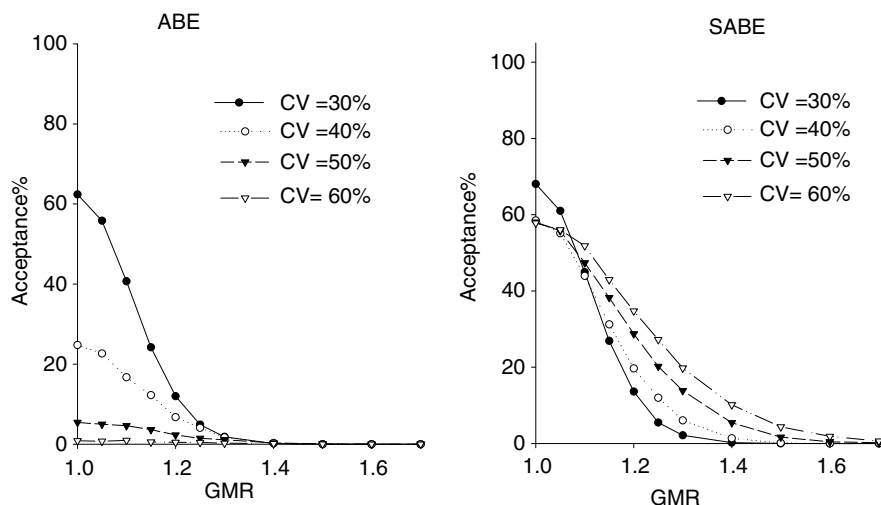


Figure 2 Percentage of simulated two-period crossover studies, with 24 subjects, in which BE was accepted by two procedures, ABE and SABE, respectively. The effect of various within-subject variations is presented (CV = 30, 40, 50, and 60%). The curves show the decreasing proportion of accepting BE as the true GMR rises. At GMR = 1.0 the two formulations are truly bioequivalent. The complement of the fraction of accepted studies is the producer risk. When SABE is used, the producer risk is about 40%, independently of the CV when it is over 30%. In contrast, when ABE is applied, the proportion of accepted studies sharply decreases, and the producer risk rises, with increasing within-subject variation. *Abbreviations:* BE, bioequivalence; ABE, unscaled average bioequivalence; SABE, scaled average bioequivalence; GMR, geometric mean ratio; CV, coefficient of variation.

BE, and at CV = 30% half of the simulated trials are evaluated with ABE and not with SABE. For the same reason, the power curves obtained by the two procedures (ABE and SABE) are similar at CV = 30%.

An important conclusion from Figure 2 is that the proposed SABE criterion of $\theta_s = 0.759$ is quite demanding, requiring more than 24 volunteers in order to achieve 90% or even 80% power. There are two ways to increase statistical power; firstly, by raising the number of volunteers and secondly by performing a replicate design study with three or four periods.

Following the first alternative, Figure 3 shows that 48 volunteers are sufficient to demonstrate scaled BE at any within-subject coefficient of variation, with a power of over 90% (i.e., a producer risk of less than 10%) if the two formulations are actually bioequivalent. This is in contrast to the low power of unscaled ABE. When the within-subject CV is higher than 40% then even 48 volunteers could not guarantee a power of 90%. Figures 4

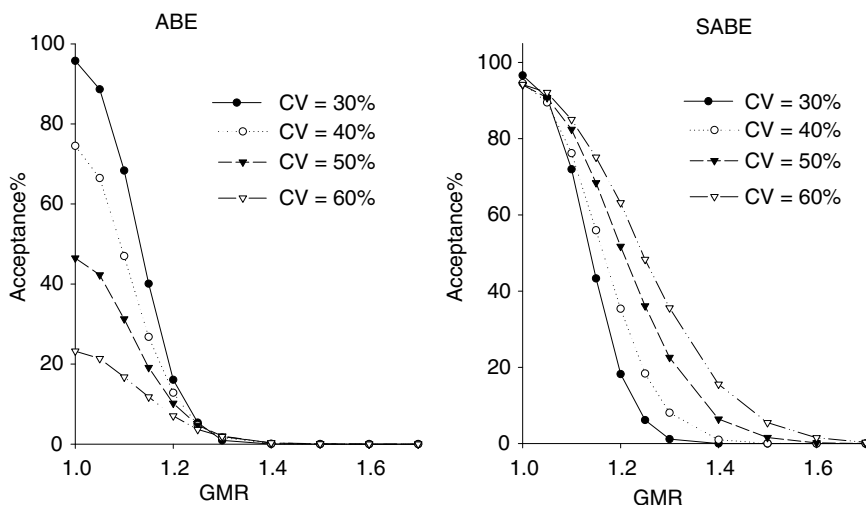


Figure 3 Percentage of simulated two-period crossover studies, with 48 subjects, in which BE was accepted by two procedures, ABE and SABE, respectively. The diagrams are similar to Figure 2 except that here twice as many subjects are assumed. By applying SABE, at GMR = 1.0, the statistical power is about 93%, and the producer risk about 7%, independently of the CV when it is over 30%. *Abbreviations:* BE, bioequivalence; ABE, unscaled average bioequivalence; SABE, scaled average bioequivalence; GMR, geometric mean ratio; CV, coefficient of variation.

and 5 show the results of simulations with three- (Fig. 4) and four-period (Fig. 5) studies. Patterson and Jones (42) note that, as was also stated earlier, the power for ABE depends on the total degrees of freedom and a four-period investigation with $N/2$ volunteers has practically the same power as a two-period study with N subjects. Figures 4 and 5 illustrate that this statement is true also for SABE. In order to reach the required 90% power, 36 volunteers are needed in three-period and 24 volunteers are required in four-period studies.

Relationship Between ABE and SABE

ABE has for many years, been the principal tool of regulation and has served as a yardstick by which any new decision criterion should be compared. As noted earlier, by rearranging the definition of SABE [Equation (5)], a relationship similar to ABE is obtained but with the BE limit increasing in proportion with the intra-subject variation:

$$-\theta_A = -\theta_S \sigma_W \leq \mu_T - \mu_R \leq \theta_S \sigma_W = \theta_A \quad (14)$$

It has also been observed that evaluating SABE in this way is statistically incorrect even though when N is fairly high ($N > 30$) then the error of

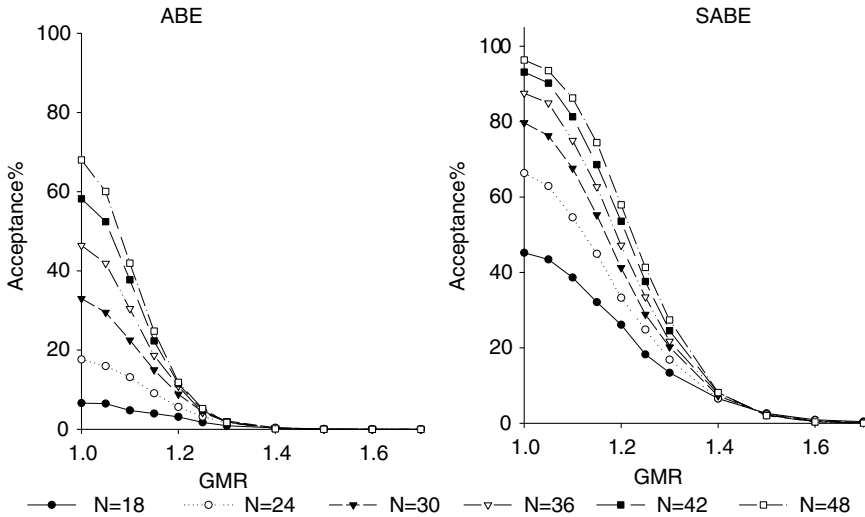


Figure 4 Percentage of simulated three-period crossover studies, with within-subject variation of $CV = 50\%$, in which BE was accepted by two procedures, ABE and SABE, respectively. Different numbers of subjects were assumed, $N = 18, 24, 30, 36, 42$, and 48 . With about 36 subjects, the statistical power of a three-period investigation is about 90% when $GMR = 1.0$. *Abbreviations:* BE, bioequivalence; ABE, unscaled average bioequivalence; SABE, scaled average bioequivalence; GMR, geometric mean ratio; CV, coefficient of variation.

evaluation is very small. Nevertheless, Equations (1) and (14) are suitable for comparing the two approaches.

Figure 1 shows that with ABE, the BE limits around the difference $\mu_T - \mu_R$ have a width of $2\theta_A$. Therefore, the limits increase linearly with the variation of σ_W . This linear relationship is depicted in Figure 1 with dashed lines in their early phase, which have slopes of $\pm\theta_S$. This permits an easy conversion between SABE and ABE. If, for instance, θ_S is set to 0.760, and with a within-subject $CV = 40\%$, then SABE behaves as ABE with a BE limit of $\pm \exp(0.385 \cdot 0.760) = (0.747 \text{ and } 1.339)$. Similarly if the $CV = 50\%$ then SABE behaves as ABE with a regulatory limit of $\pm \exp(0.472 \cdot 0.760) = (0.699 \text{ and } 1.431)$.

Consequently, at variations of 40 and 50%, SABE corresponds to the unscaled ABE with BE limits of (0.75 and 1.33) and (0.70, 1.43), respectively. This illustrates that if the regulatory strategy of stepwise widening of the BE limits is followed (see earlier) then, for instance, regulatory limits between 0.75 and 1.33 could be satisfactory when the variation is 40% but not when it is higher.

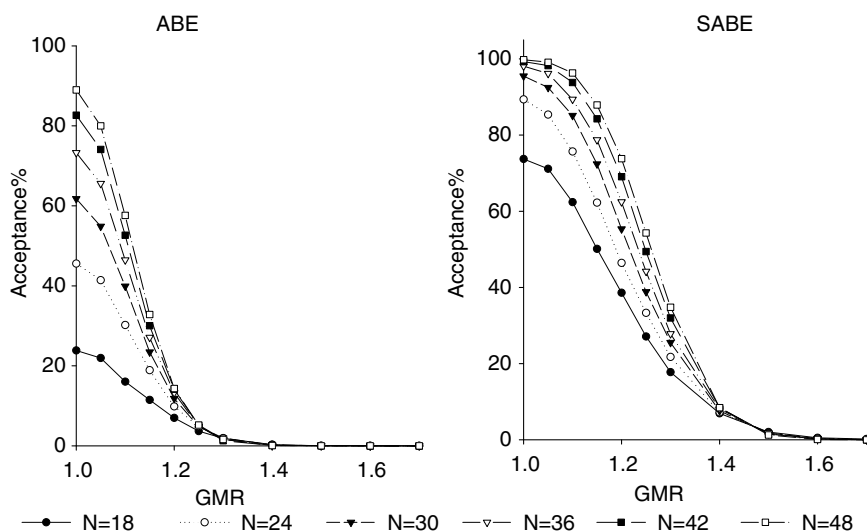


Figure 5 Percentage of simulated four-period crossover studies, with within-subject variation of $CV = 50\%$, in which BE was accepted by two procedures, ABE and SABE, respectively. Different numbers of subjects were assumed, $N = 18, 24, 30, 36, 42$, and 48 . With about 24 subjects, the statistical power of a four-period investigation is about 90% when $GMR = 1.0$. *Abbreviations:* BE, bioequivalence; ABE, unscaled average bioequivalence; SABE, scaled average bioequivalence; GMR, geometric mean ratio; CV, coefficient of variation.

Statistical Controversies and Unresolved Issues

Questioning the Assumptions

The statistical models underlying the calculations make several assumptions. Relaxation and modifications of these assumptions can lead to different analyses and results. The issue of the multiplicity of models is particularly troublesome when parameters are estimated from replicate designs (with three or more periods) because each model corresponds to a different statistical and computational procedure, and different final estimates and conclusions. BE studies are not powered sufficiently to check the validity of model assumptions. In the case of simple 2×2 BE studies, the data are analyzed in a straightforward manner and the computational procedure is simple and unique. However, theoretically, the model assumptions cannot be checked.

The Assumption of Common Between-Subject

Variation: $\sigma_{BT} = \sigma_{BR}$

At first sight, this assumption does not have a role in the calculation of the regulatory decision criterion. However, the impression is incorrect. If this

assumption is relaxed then the formula for the standard error of the difference will contain a variance component for the so-called subject-by-formulation interaction.

Subject-by-formulation interaction is not an issue in the case of the 2×2 design. The standard error calculated from residual mean square from the analysis of variance (ANOVA) table is correct regardless of its interpretation.

However, as Schuirmann (61) pointed out, this is not true for designs with three or more periods. In these studies, the standard error of $(\mu_T - \mu_R)$ quite often cannot be simply estimated from the ANOVA table. To solve the problem, Schuirmann (61) suggested that the method of restricted maximum likelihood be applied, for instance by using Proc Mixed of the SAS program (62). However, Proc Mixed has numerous options, and even the statistical model can be written in different ways (41,42). The suggestion here is that replicate-design studies should be evaluated with a mixed-effect modeling approach even though, because of the richness of the model, slightly different statistical estimates can be obtained. This can be quite problematic in borderline cases. Hsuan and Reeve (63) described an alternative and unique ANOVA-type of estimation method.

The Assumption of Common Within-Subject

Variation: $\sigma_{WT} = \sigma_{WR}$

It is generally assumed that the test and reference drug formulations have the same within-subject variability. However, this assumption is not always correct. For example, a reference formulation of nadolol was found to have high intraindividual variation ($CV = 50\%$ and 39% for $C_{\max}$ and AUC, respectively) (11). In contrast, a test product showed much lower variation (26% and 19% for $C_{\max}$ and AUC, respectively) (11). In such cases, we cannot talk about a HV drug but only about a HV drug product (11,44). Therefore, it could be useful to evaluate whether the within-subject variations of the two drug formulations are the same. The comparison of the ratio between the variances could be based on a significant F -statistic. A significant F -value would indicate that the two drug products are *not* bioequivalent; consequently, it would be pointless to proceed with the comparison of the means.

Such sequential tests, first between variances and then between means, have been recommended in the context of individual BE (64,65). It would have to be determined whether such a test would be helpful to regulatory authorities also when ABE is evaluated. The magnitude of the ratio for regulatory decision would also have to be determined.

The Assumption of Normality: Logarithmic Metrics
Have Normal Distributions

The most serious violation of the normality assumption is observed if the results contain one or more outliers. In the case of unscaled ABE, outliers

always increase the producer risk, and so it is not surprising that statisticians on the producers' side try to remove them, and that this attempt is regarded with suspicion from the regulatory side.

In terms of outliers, SABE is very different from ABE. First of all, outliers increase both the numerator and the denominator of the scaled difference $(\mu_T - \mu_R)/\sigma_W$, and therefore the effect of outliers is limited. But more importantly, if the results contain outliers then they could falsely classify the study as an HV case, thereby leading to its evaluation by SABE. The roles in the game between producers and regulatory agencies would be reversed such that it could be in the interest of the producers to retain outliers whereas the regulators may want to remove them. Statistical tools are available to handle this scenario; for example, the scale factor could be estimated by a robust method such as the mean absolute deviation. Regulatory reviewers are aware of this problem and concerned about it and therefore statistical methods and appropriate software tools need to be developed to remedy this concern.

Constraint on the GMR

If SABE is used for HV drugs then it is possible that BE is claimed even though the estimated GMR is outside the interval between 0.80 and 1.25. A similar situation has been noted when individual BE was considered (see earlier) and is a cause of some concern. To remedy this problem, a second regulatory criterion has been proposed (8). This expects that BE can be claimed only if the criterion for individual BE was satisfied and, in addition, the estimated GMR was between 0.80 and 1.25.

The concern about the possibility of large point estimates of GMR has also been expressed in connection with the determination of BE for HV drugs (66). However, concern is much less justified in this case for two reasons. First, the range of large estimated GMR values is much higher in the case of individual BE than with HV drugs (31).

Second, and more importantly, with individual BE, large GMR values are due to the assumed model [Equation (3)] and the suggested procedure of its evaluation (27,28,32). In contrast, with HV drugs, large GMR values occur naturally, as a consequence of the high intrasubject variability. Any constraint on the GMR's amounts to truncating the normal distribution of the difference between the logarithmic means. It is often assumed that the logarithmic means as well as their differences (i.e., the logarithm of GMR) have normal distribution. Consequently, any constraint on the GMRs amounts to truncating the normal distribution of the difference between the logarithmic means.

Statistically, the constraint criterion on GMR is quite strange as it corresponds to an ABE test with a 50% CI. Also, statistical testing of hypotheses that are not independent is analogous to a legal double jeopardy. Still, it can be argued that incorporation of the constraint into a possible

SABE regulatory guideline could be acceptable. The strongest argument is that it has a track record, since as noted earlier. Health Canada has used this criterion for $C_{\max}$ for a number of years with success and without any serious concern from the medical community (12) or other significant repercussions.

It is interesting to note the effect of the GMR constraint on the overall performance, notably on the producer risk, of BE determination. This effect depends on the comparative positions of the two criteria: the GMR constraint and the regulatory limit for SABE. When, this regulatory limit is $\theta_S = 1.00$ (corresponding to a switching variability of $\ln(1.25)$ as suggested by Boddy et al. (29)) then the GMR constraint can become the prominent component of the joint regulatory criteria (67). However, as our additional simulations demonstrate, when the regulatory limit for SABE is set to $\theta_S = 0.760$ then the GMR constraint had little effect on the combined performance of the BE test. In this case, the proposed BE limit of $\theta_S = 0.760$ is a stricter criterion than the GMR constraint. In order to improve some of the unfortunate properties of the GMR restrictions, Karalis et al. (68,69) proposed a combined regulatory criterion, which allows testing by SABE with a GMR constraint in a single step. However, the feasibility of this method still needs some support.

SUMMARY AND RECOMMENDATIONS

The determination of BE of highly variable drugs has been a difficult problem. The approach of ABE has usually been applied; it typically expects that the 90% CI around the estimated ratio of geometric means of the drug products be between 0.80 and 1.25. It is very difficult to satisfy this criterion in the case of highly variable drugs unless an unreasonably large number subjects is included.

Various approaches have been proposed to alleviate of this problem. They include:

1. the relaxation of a regulatory requirement,
2. the stepwise widening of the BE limits,
3. the utilization of steady-state studies,
4. the application of individual BE,
5. the continuous expansion of the BE limits, and
6. the evaluation of scaled ABE.

The approach of scaled ABE is particularly useful for determining the BE of highly variable drugs. It has relevant interpretations in terms of, for instance, therapeutic switchability, distance metrics, and the equivalence of clinical effect sizes. With highly variable drugs, much higher statistical power is obtained by scaled than by unscaled ABE. Scaled ABE yields about 90% power with 48, 36, and 24 subjects in 2-, 3-, and 4-period investigations, respectively, when the true ratio of geometric means = 1.0.

NOTATIONS AND ABBREVIATIONS

The following notations and abbreviations are used in this paper. The response variable is a logarithmically transformed BE metric ($\log AUC$ or $\log C_{\max}$).

α	Probability
AUC	Area under the curve contrasting plasma concentration and time
$C_{\max}$	Maximum plasma concentration
CL	Confidence limit calculated for scaled average bioequivalence, Eq. (13)
CV	Coefficient of variation
μ_T and μ_R	Means of the test and reference formulations
m_T and m_R	Estimated means of the test and reference formulations
N	Number of volunteers
S	Number of study sequences
SE	Standard error
σ_0	Switching variation, defines variabilities of HV drugs; $\sigma_0 = 0.294$ is suggested
σ^2	Variance; also used as the denominator in Eq. (3) for individual BE
σ_{BT}^2 and σ_{BR}^2	Between-subject variances of the test and reference formulations
σ_D^2	Variance component for subject-by-formulation interaction
σ_{Res}^2	Residual variance in 2-period BE studies; it contains the within-subject variance
s_{Res}^2	Estimated residual variance in 2-period BE studies; it is calculated as residual variance term in an analysis of variance
σ_W^2	Variance related to the within-subject variance; also used as the denominator in Eq. (5) for scaled average BE
s_W^2	Estimated variance related to the within-subject variance
σ_{WT}^2 and σ_{WR}^2	Within-subject variances of the test and reference formulations
s_{WT}^2 and s_{WR}^2	Estimated within-subject variances of the test and reference formulations
θ_A	Regulatory BE limit for average BE, usually $\log(1.25)$
θ_S	Regulatory BE limit for scaled average BE; 0.760 is the suggested value

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Statistical Considerations: Alternate Designs and Approaches for Bioequivalence Assessments

Sanford Bolton

University of Arizona, Tucson, Arizona, U.S.A.

Charles Bon

Biostudy Solutions LLC, Wilmington, North Carolina, U.S.A.

PARALLEL DESIGN IN BIOEQUIVALENCE STUDIES

The great majority of bioequivalence (BE) studies measure drug concentrations in body fluids, such that products can be compared within an individual using crossover designs. In some rare circumstances, this approach is either not possible or impractical. For example, drugs with long half-lives may not be amenable to a crossover design or studies where a clinical endpoint is required in patients because of insufficient blood concentrations. In these cases a parallel design may be used.

In parallel designs comparative products are not given to the same patient. Patients are randomly assigned to one of the products under investigation. In this discussion, examples are used where two products are compared to each other, a Test and Reference product. Typically, a random device is used to assign product to patients as they enter the study, with an aim of having equal numbers of patients in each product group. For a BE study, it would be expected that patients would all be entered together, with each patient assigned a unique number. If more patients are needed than can be accommodated at one site, a multi-center study may be necessary. Randomization schemes for parallel studies have been described in the literature (1). Note that for these designs, the number of observations in each group does not need to be identical; drop-outs do not invalidate any of the remaining data.

Endpoints in clinical studies can be “continuous” data or discrete. For example, the endpoint could be treadmill time to angina, or as for a local treatment for ulcers, the endpoint can be dichotomous, i.e., success or failure. The analysis of both kinds of studies will be discussed.

Another problem with parallel studies is how to construct a statistical test comparing products. For numerical data, one should consider whether or not to transform the data. The usual BE study uses a log transform of the pharmacokinetic parameters. In clinical studies, it is not obvious if the clinical result should be transformed. In general, a transformation is not necessary, but may depend on the nature of the resulting data. For dichotomous data, there is a different problem when comparing outcomes.

The analysis will be illustrated using the following hypothetical data.

Consider a drug taken orally that is absorbed, but results in such low drug concentrations in the blood that an acceptable analysis is not available. Such a study thus requires a clinical endpoint that can be measured objectively. Suppose that the drug is given once daily for seven days. The endpoint is the average time it takes for patients to fall asleep. A parallel study is used because of the potential for carryover of a physiological or psychological nature. At first, the data are considered to be approximately normal, and no transformation is needed. The study design is single blind, with the evaluator being blinded, as is typical for the usual BE crossover studies. The results of the study are as follows:

Product	<i>N</i>	Average (hour)	Variance
Test	24	0.980	0.228
Reference	26	0.949	0.213

Without a (log) transformation, the confidence interval (CI) computation is more complicated than that for the usual crossover design with a log transformation. The ratio of Test/Reference is not normally distributed. Before the log transformation requirement was initiated, an approximate CI was computed as described by Food and Drug Administration (FDA) and the literature (1). However, presently, in many circumstances where log transformation is not appropriate, the FDA recommends the use of Fieller’s method (2) for computing CIs. The CI will be calculated using both of these methods to illustrate the methods and to compare the results.

Old FDA Method

$$CI = \frac{[(\text{Average test} \pm t(\text{d.f.}, 0.1) * \text{sqrt}(\text{variance} * (1/N_1 + 1/N_2))]}{\text{Average reference}},$$

where the $t(\text{d.f.}, 0.1)$ value is from the t distribution with appropriate degrees of freedom at the (one-sided) 5% level. The variance, in this case would be

the pooled variance from the two groups. The computations for the numerator are the same as that computed for a 90% CI in a two-independent-group t test.

In this example, the point estimate (Test/Reference) is 1.033 with lower and upper 90% CI limits equal to 0.923 and 1.143, respectively (See [Table 1](#) for raw data and calculations).

One could also use a $\log_e$ transformation, if appropriate, provided that the rationale for using a transformation is justified. Using a $\log_e$ transformation, the point estimate is 1.031 with lower and upper 90% CI limits equal to 0.919 and 1.158, respectively (See [Table 2](#) for raw data and calculations). These values are similar to those for the untransformed data. The calculations are:

Point estimate for Test = $\text{Exp}_{(\text{Average Test})}$

Point estimate for Reference = $\text{Exp}_{(\text{Average Reference})}$

T/R ratio = $\text{Exp}_{(\text{Average Test} - \text{Average Reference})}$

CI = $\text{Exp}_{[(\text{Average Test} - \text{Average Reference}) \pm t(\text{d.f., } 0.1) * \text{sqrt}(\text{variance} * (1/N_1 + 1/N_2))]$,

where Exp is the antilog_e function and CI is the Confidence Interval.

Fieller's Method

Fieller's method (2) can be used to compute CIs for the ratio of two normally distributed variables. There are assumptions when using Fieller's method that include the assumption of normality. Also the value of the denominator in Fieller's equation must show the Reference product average to be "statistically significant" when compared to zero. In most cases, the results of this approach should give similar conclusions as the old FDA method above.

The method is described for paired data in an FDA document (3), which is duplicated below.

Fieller's Calculation for Paired Data (Correlated Values)

For an example of this calculation, see Ref. 3.

$$[(T/R) - G(\sigma - RT/\sigma - RR) \pm (t/R) * \text{Sqrt}(K * \sigma - RR/n)] / (1 - G)$$

$$G = t^2 * \sigma - RR / (n * R^2)$$

$$K = (T/R)^2 + (\sigma - TT/\sigma - RR) * (1 - G) + (\sigma - RT/\sigma - RR) * [G * (\sigma - RT/\sigma - RR) - 2 * (T/R)]$$

$\sigma - TT$ = Variance Test

$\sigma-RR$ = Variance Reference

$$\sigma-RT = [\Sigma(Ti-T)(Ri-R)]/(n-1)$$

Ti = i th Test value

Ri = i th Reference value

T =Average test

R = Average reference

t = Critical t -distribution value with $n - 1$ degrees of freedom.

Table 1 Data for Parallel Design Study (Clinical Endpoint)

Subject	Test	Subject	Reference
1	0.82	1	0.83
2	0.54	2	1.22
3	1.01	3	1.14
4	1.40	4	0.88
5	0.89	5	0.95
6	1.00	6	1.40
7	0.76	7	1.10
8	1.23	8	0.84
9	0.87	9	0.99
10	0.99	10	0.61
11	1.10	11	0.68
12	1.15	12	1.03
13	0.76	13	0.79
14	0.65	14	1.09
15	1.25	15	0.91
16	1.11	16	1.22
17	0.77	17	1.10
18	0.63	18	0.89
19	0.98	19	1.17
20	1.32	20	0.58
21	1.26	21	1.11
22	0.94	22	0.75
23	0.99	23	0.95
24	1.11	24	1.03
		25	0.88
		26	0.54
	<i>Test</i>		<i>Reference</i>
Average	0.980417		0.949231
Standard deviation	0.228197		0.213353
Variance	0.052073		0.045519
Point estimate =	1.032853863		

(Continued)

Table 1 Data for Parallel Design Study (Clinical Endpoint)
(Continued)

Subject	Test	Subject	Reference
$t =$	1.677224191		
Pooled variance =	0.048660009		
Upper level	1.143170257		
Lower level	0.922537469		

Table 2 Data for Parallel Design Study Transformed

Subject	Test	Log _e	Subject	Reference	Log _e
1	0.82	-0.19845	1	0.83	-0.18633
2	0.54	-0.61619	2	1.22	0.19885
3	1.01	0.00995	3	1.14	0.13103
4	1.40	0.33647	4	0.88	-0.12783
5	0.89	-0.11653	5	0.95	-0.05129
6	1.00	0.00000	6	1.40	0.33647
7	0.76	-0.27444	7	1.10	0.09531
8	1.23	0.20701	8	0.84	-0.17435
9	0.87	-0.13926	9	0.99	-0.01005
10	0.99	-0.01005	10	0.61	-0.49430
11	1.10	0.09531	11	0.68	-0.38566
12	1.15	0.13976	12	1.03	0.02956
13	0.76	-0.27444	13	0.79	-0.23572
14	0.65	-0.43078	14	1.09	0.08618
15	1.25	0.22314	15	0.91	-0.09431
16	1.11	0.10436	16	1.22	0.19885
17	0.77	-0.26136	17	1.10	0.09531
18	0.63	-0.46204	18	0.89	-0.11653
19	0.98	-0.02020	19	1.17	0.15700
20	1.32	0.27763	20	0.58	-0.54473
21	1.26	0.23111	21	1.11	0.10436
22	0.94	-0.06188	22	0.75	-0.28768
23	0.99	-0.01005	23	0.95	-0.05129
24	1.11	0.10436	24	1.03	0.02956
			25	0.88	-0.12783
			26	0.54	-0.61619
	<i>Test</i>				<i>Reference</i>
Average	-0.04777				-0.07852
Point					0.92448
Estimate =	0.95335				
$t =$	1.677224				
Pooled					
Variance =	0.05942				
Upper level	1.15775			(log _e) 0.146479	
Lower level	0.918533			(log _e) -0.08498	

Fieller’s Calculation for Independent Data

If the two groups are independent as in the above example, the term that relates to the correlation of the data for the two groups, $\sigma - RT$, is considered to be zero, and the equations simplify considerably:

nt = Number of subjects on the Test

nr = Number of subjects on the Reference

$$\text{Interval} = [(T/R) \pm (t/R) * \text{Sqrt}((\sigma - TT/nt) + (T/R)^2 * (\sigma - RR/nr) - G * (\sigma - TT/nt))] / (1 - G)$$
$$G = t^2 * \sigma - RR / (nr * R^2)$$

Applying these equations to the data in [Table 1](#) without a transformation, the results are as follows:

	Test	Reference
Average	0.980417	0.949231
n	24	26
Variance	0.052074	0.045519
$T/R = 1.032854$		
$G = 0.0054659$		
Upper interval = 1.151679		
Lower interval = 0.926170		

Schuirmann (4) simplified this calculation further, using what is termed the Fixed Fieller’s calculation. If $\sigma - TT = \sigma - RR$, then an Analysis of Variance can be calculated, including whatever terms are important based on the study design (e.g., terms for Treatment, Center, Gender, etc), and then the Mean Square term for Error is used in place of both $\sigma - TT$ and $\sigma - RR$. In the example above, this calculation would involve using the pooled variance, which is 0.04866, in place of $\sigma - TT$ and $\sigma - RR$. Doing this, G becomes 0.005843 and the lower and upper CI limits are 0.925995 and 1.151066, respectively.

OUTLIERS

An outlier is an observation far removed from the bulk of the observations. A more detailed discussion and statistical detection of outliers, as well as their treatment can be found in a number of references (1).

For crossover studies and parallel studies, the detection of an outlier using common statistical methods is straightforward. Using an appropriate

statistical model, a single statistical outlier can be identified. Although this alone may be sufficient to suspect an anomaly, usually it would be more definitive if other evidence is available to verify that the suspected datum is indeed “mistaken.” A more creative approach is possible in the case of replicate designs (see below). In these situations, the estimates of within subject variability can be used to identify outliers. For example, if the within subject variance for a given treatment is 0.61, but reduces to 0.04 when omitting the subject with the suspected outlier value, an F test can be performed comparing variances for the suspect data and the remaining data. The F ratio, in this example, is:

$$F = 0.61/0.04 = 15.3$$

The degrees of freedom for the numerator are those for the variance estimate obtained using the results from all subjects and those for the denominator are those for the variance estimate obtained from the results omitting the suspected outlier. In the above example, if the numerator and denominator degrees of freedom were 30 and 28, respectively, then an F value of 15.3 is highly significant ($P < 0.01$). One may wish to correct the significance level, although there is no precedent for such an approach. An alternative analysis could be an analysis of variance (ANOVA) with and without the suspected outlier. An F test with 1 DF in the numerator and appropriate degrees of freedom in the denominator would be:

$$[\text{Error SS (all data)} - \text{Error SS (without outlier data)}]/[\text{Error SS (all data)}/\text{d.f.}],$$

where d.f. is the degrees of freedom when all of the data are analyzed.

Note that Error SS/d.f. is the Error Mean Square.

Another approach that has been used is to compare results for periods 1 and 2 *versus* periods 3 and 4 in a 4 period fully replicated design.

Of course, if there is an obvious cause for the outlier, a statistical justification is not necessary. However, further evidence, even if only suspicious, is helpful.

If an outlier is detected, as noted above, the most conservative approach is to find a reason for the outlying observation, such as a transcription error, or an analytical error, or a subject that violated the protocol, etc. In these cases, the data may be reanalyzed with the corrected data, or without the outlying data if due to analytical or protocol violation, for example.

If an obvious reason for the outlier is not forthcoming, one may wish to perform a new small study, replicating the original study, including the outlying subject along with a number of other subjects (at least 5 or 6) from the original study. The results from the new study can be examined to determine if the data for the outlier from the original study is anomalous. It should be noted that the data from the small study are not used as a

replacement for any of the original data, but serve only to confirm, or refute, that the suspected outlier subject is reproducibly an outlier. The procedure here is not fixed, but should be reasonable, and make sense. One can compare the test to reference ratios for the outlying subject in the two studies, and demonstrate that the data from the new study shows the outlying subject is congruent with the other subjects in the new study, for example.

DICHOTOMOUS OUTCOME

Studies with a dichotomous outcome (e.g., cured or not cured) are, typically, clinical studies on patients. They may be parallel or crossover studies. An example of a crossover study with a dichotomous outcome would be an application of a patch or topical product studying sensitivity or evidence of a pharmacodynamic response. It would be difficult to compare products based on a ratio for crossover designs with a dichotomous outcome. Statistical tests for such designs would fall in the category of a McNemar test, where only those results that are different for the two products are considered in the analysis. Thus, the results which are “positive” for both products, or “negative” for both products would not be considered in the analysis. Thus far, no regulatory requirements have been issued for BE for such designs.

Parallel designs for BE using dichotomous outcomes are not uncommon. These studies usually use patients with the “disease.” The results are analyzed using either the binomial distribution or the normal approximation to the binomial, where the outcome may be cured or not cured. The FDA suggests that the 90% CI for the difference of the proportion of “successes” (or “failures”) between the products be within ± 0.20 for equivalence. Some criteria may be based on a one-sided 95% CI in the case of noninferiority studies. Proposals have been made to modify the ± 0.20 window for equivalence depending on the observed proportion (4), though at the time of this writing this has not been applied to BE studies.

Consider the following example:

Test product : 160/200 successes = 0.80

Reference product : 170/200 successes = 0.85

The CI for the difference in proportion of successes is calculated as follows:

$$90\% \text{ CI} = (0.80 - 0.85) \pm [\text{sqrt}((P1 * Q1/N1) + (P2 * Q2/N2)) \\ + 0.5 * (1/N1 + 1/N2)]$$

$$\begin{aligned}
&= -0.05 \pm [1.645 * \text{sqrt}((0.8 * 0.2/200) + (0.85 * 0.15/200)) \\
&\quad + 0.5 * (1/200 + 1/200)] \\
&= -0.05 \pm 0.0674 = -0.117 \text{ to } 0.0174
\end{aligned}$$

This result would pass the ± 0.20 BE requirement (i.e., the CI on the difference is completely contained within the regulatory limits of -0.20 to $+0.20$).

STEADY STATE STUDIES

Steady state (SS) studies have been used to study BE for some drug products, e.g., controlled release and highly variable drug products. SS is approximately attained after about 5 to 7 drug half-lives. For example, if the half-life is 8 hours, the drug should be administered for 40 to 56 hours; for example, five to seven single doses administered at 8 hour intervals. At SS, theoretically, $C_{\max}$, $C_{\min}$ and the AUC during a dosing interval remain constant. In particular, the relative amount of drug absorbed is measured by the area under the curve (AUC) over the dosing interval at SS. SS studies are now discouraged by the FDA for BE determinations (6). One reason given for this proposal is that due to the accumulation of drug, differences between products are minimized, especially for $C_{\max}$. As an example, assume drug A, with an 8 hour half-life is given as a single dose every 8 hours for 3 days. Further assume that the $C_{\min}$ concentration (the drug concentration immediately before administration of the next dose) is 10 ng/mL for both Test and Reference products. If the time to peak for both products is 2 hours after dosing, then the accumulated value of drug still present at this time from previous doses is $C_{\min} * \text{Exp}(-2 * 0.693/8) = 10 * \text{Exp}(-2 * 0.086625) = 8.4 \text{ ng/mL}$. If the Test product dose actually produces a $C_{\max}$ above the accumulated level that is equal to 8 ng/mL and the Reference product produces a $C_{\max}$ of 12 ng/mL ($T/R = 8/12 = 0.67$) the observed $C_{\max}$ values at SS would be $8.4 + 8 = 16.4 \text{ ng/mL}$ for the Test product and $8.4 + 12 = 20.4 \text{ ng/mL}$ for the Reference product ($T/R = 16.4/20.4 = 0.80$). Clearly, the SS $C_{\max}$ masks the true difference between the Test and Reference product.

However, in the SS study, variability is usually also reduced which gives a greater statistical sensitivity to detect a difference between products. If the accumulation is not great, the SS study could be useful from a practical point of view to compare highly variable drug products. Thus, there is some controversy about the use and utility of SS studies.

SS studies are typically crossover studies with multiple dosing. Two groups of patients are entered into the study similar to the usual two-treatment, two-period design. However in the SS design, multiple doses are

administered, using the usual dosing schedule, for a sufficient period of time to attain SS. One would estimate the total number of doses needed based on half-life information in the package insert, literature or available experimental results.

SS is achieved when the pharmacokinetic parameters remain constant with a given multiple dosing regimen. Typically, dosing should be administered for at least 5 to 7 half-lives with the last three or more consecutive doses being at the steady-state condition. Appropriate dosage administration and sampling should be carried out to document SS. The trough concentration data should be analyzed statistically to verify that SS was achieved prior to period 1 and period 2 sampling.

According to the FDA Guidance (6), the following parameters should be measured:

1. individual and mean blood drug concentration levels,
2. individual and mean trough levels ($C_{\min\text{SS}}$)
3. individual and mean peak levels ($C_{\max\text{SS}}$)
4. calculation of individual and mean steady-state $\text{AUC}_{\text{interdose}}$ ($\text{AUC}_{\text{interdose}}$ is AUC during a dosing interval at steady-state)
5. individual and mean percent fluctuation

$$[= 100 * (C_{\max\text{SS}} - C_{\min\text{SS}}) / C_{\text{average}}],$$

where $C_{\text{averageSS}} = \text{AUC}_{\text{interdoseSS}} / \text{Dosing interval}$

6. individual and mean time to peak concentration.

The log-transformed $\text{AUC}_{\text{interdose}}$ and $C_{\max\text{SS}}$ data during the final dosing interval should be analyzed statistically using analysis of variance. The 90% CI for the ratio of the geometric means of the pharmacokinetic parameters ($\text{AUC}_{\text{interdose}}$ and $C_{\max\text{SS}}$) should be within 80% to 125%. Fluctuation for the Test product should be evaluated for comparability with the fluctuation of the Reference product.

BE STUDIES PERFORMED IN GROUPS

BE studies are usually performed at a single site, where all subjects are recruited and studied as a single group. On occasion, more than one group is required to complete a study. For example, if a large number of subjects are to be recruited, the study site may not be large enough to accommodate the subjects. In these situations, the study subjects may be divided into two cohorts. Each cohort is used to assess the comparative products individually, as might be done in two separate studies. Typically, the two cohorts are of approximately equal size. The final assessment is based on a combination of both groups. The totality of data is analyzed with a new term in the

ANOVA, a Treatment-by-Group interaction term. This is a measure (on a log scale) of how the ratios of Test-to-Reference differ in the groups. For example, if the ratios are very much the same in each group, the interaction would be small or negligible. If interaction is large, as tested in the ANOVA, then the groups statistically should not be combined. However, if at least one of the groups individually pass the CI criteria, then the Test product might be acceptable. If interaction is not statistically significant ($P > 0.10$), then the CI based on the pooled analysis, after dropping the interaction term, will determine acceptability. It is an advantage to pool the data, as the larger number of subjects increases power and there is a greater probability of passing the BE CI, if the products are truly bioequivalent.

An interesting question arises if more than two groups are included in a BE study. As before, if there is no interaction, the data should be pooled. If interaction is evident, it is implied that at least one group is different from the others. Usually, it will be obvious which group is divergent from a visual inspection of the treatment differences in each group. The remaining groups may then be tested for interaction. Again, as before, if there is no interaction, the data should be pooled. If there is interaction, the aberrant group may be omitted, and the remaining groups tested, and so on. In rare cases, it may not be obvious which group or groups are responsible for the interaction. In that case, more statistical treatment may be necessary, and a statistician should be consulted. In any event, if any single group or pooled groups (with no interaction) passes the BE criteria, the test should pass. If a pooled study passes in the presence of interaction, but no single study passes, one may still argue that the product should pass, if there is no apparent reason for the interaction. For example, if the groups are studied at the same location under the identical protocol, and there is overlap in time among the treatments given to the different groups, as occurs often, there may be no obvious reason for a significant interaction. Perhaps, the result was merely due to chance. One may then present an argument for accepting the pooled results.

The following statistical models have been recommended for analysis of data in groups:

Model 1: GRP SEQ GRP*SEQ SUBJ(GRP*SEQ) PER(GRP) TRT
GRP*TRT.

If the GRP*TRT term is not significant ($P > 0.10$), then re-analyze the data using Model 2.

Model 2: GRP SEQ GRP*SEQ SUBJ(GRP*SEQ) PER(GRP) TRT,
where

GRP = Group

SEQ = Sequence

GRP*SEQ = Group-by-Sequence

SUBJ(GRP*SEQ) = Subject nested within Group-by-Sequence

PER(GRP) = Period nested within Group

TRT	= Treatment
GRP*TRT	= Group-by-Treatment interaction

REPLICATE STUDY DESIGNS

Replicate studies in the present context are studies in which individuals are administered one or both products on more than one occasion. For purposes of BE, either three or four period designs are recommended. The two-treatment-four-period design is the one most used. FDA (1) gives sponsors the option of using replicate design studies for all bioequivalence studies. Replicate studies may provide information on within subject variance of each product separately, as well as potential product-by-subject interactions, although these analyses are not required by FDA.

The FDA recommends that submissions of studies with replicate designs be analyzed for average BE. The following (Table 3) is an example of the analysis of a two-treatment, 4 period replicate design to assess average BE. The design has each of two products, balanced in 2 sequences, ABAB and BABA, over four periods. Table 3 shows the results for C_{max} for a replicate study. Eighteen subjects were recruited for the study and 17 completed the study. An analysis using the usual approach for the two-treatment, two-period design, as discussed above, is not recommended. The FDA recommends use of a mixed model approach as in SAS PROC MIXED (7). The recommended code is:

```
PROC MIXED;
CLASSES SEQ SUBJ PER TRT;
MODEL LNCMAX=SEQ PER TRT/DDFM=SATTERTH;
RANDOM TRT/TYPE=FA0(2) SUB=SUBJ G;
REPEATED/GRP=TRT SUB=SUBJ;
LSMEANS TRT;
ESTIMATE 'T VS. R' TRT 1 -1/CL ALPHA=0.1;
RUN;
```

The abbreviated output of the analysis of the Log_e(C_{max}) data for the Test (A) and Reference (B) products is shown in Table 4.

INTERIM ANALYSES

Interim analyses can be interpreted in a very broad way to cover any situation where an evaluation of results from a clinical study is conducted on data ($n < N$) that is a subset of the final data set (N), or potentially final data set, from the study. Using this broader definition, add-on designs, group sequential designs, adaptive designs, as well as the more traditional interim “look” designs, can all be considered to be interim analyses. A justification for this broader definition is that in all these approaches, because an analysis on a subset of the complete data is conducted, when (if) the complete data

set is analyzed, the probabilities differ from what they would have been had only the analysis on the complete data set been performed.

Interim Look

During the conduct of a BE trial, the sponsor of the study may want to evaluate how things are looking after some portion, usually $1/2$, of the subject samples have been analyzed. The rationale behind the interim look is that if the study appears that it will likely fail, then money and time are saved by stopping the analyses early and returning to the formulators to get an improved product. If the results look reasonable at the interim point, such that analyses should continue for all subjects, no harm has been done by taking an interim look. The reasoning behind an interim look is based on the premise that if the study (analyses) were to be continued to completion, the same statistics on the same data would be performed, regardless of whether the data were analyzed at some interim point. While on the surface this seems logical, it is not a correct assessment of the impact of the interim look.

Consider the following simplistic example:

Assume three coins were to be flipped, where heads can be expected half the time and tails can be expected half the time. The process of flipping the coins can be likened to checking subjects into the clinic for a BE study, dosing them and collecting samples for analyses. For the coin study to be successful, at least two heads must result from the three coins. This is analogous to meeting FDA's BE criteria for the 90% CI on the Test-to-Reference ratio. Because revealing the results of the flipped coins will be expensive (analyzing the samples), an interim look at the data is performed. If the first coin flipped did not produce a head, then the study may be halted and some new coins (re-formulate the product) obtained.

For three coins, there are eight equally possible outcomes with regards to heads (H) and tails (T):

HHH HHT HTH HTT THH THT TTH TTT.

Four of these (HHH, HHT, HTH and THH) represent successful studies (i.e., can be submitted to FDA). It is clear that if all three coins were flipped and the results revealed, there should be four chances of success out of eight possible outcomes for a probability of $4/8$ or 0.500 . However, an interim look at the results from coin 1 can be made to decide if the results of coins two and three should be revealed. Using this rule, the outcome THH, which is one of the four successful ones, would never be seen. As a result, there will only be three chances of success out of eight possible outcomes for a probability of $3/8$ or 0.375 . This reduction in the probability of success is true regardless of whether the analysis was stopped at the interim point or had obtained a head with the first flipped coin and then proceeded to reveal the results from the other two. By taking an interim look, the probabilities

Table 3 Results of a Four-Period, Two-Sequence, Two-Treatment, Replicate Design ($C_{\max}$)

Subject	Treatment	Sequence	Period	$C_{\max}$	$\text{Log}_e(C_{\max})$
1	Test	1	1	14.0	2.639
2	Test	1	1	16.7	2.815
3	Test	1	1	12.95	2.561
4	Test	2	2	13.9	2.632
5	Test	1	1	15.6	2.747
6	Test	2	2	12.65	2.538
7	Test	2	2	13.45	2.599
8	Test	2	2	13.85	2.628
9	Test	1	1	13.05	2.569
10	Test	2	2	17.55	2.865
11	Test	1	1	13.25	2.584
12	Test	2	2	19.8	2.986
13	Test	1	1	10.45	2.347
14	Test	2	2	19.55	2.973
15	Test	2	2	22.1	3.096
16	Test	1	1	22.1	3.096
17	Test	2	2	14.15	2.650
1	Test	1	3	14.35	2.664
2	Test	1	3	22.8	3.127
3	Test	1	3	13.25	2.584
4	Test	2	4	14.55	2.678
5	Test	1	3	13.7	2.617
6	Test	2	4	13.9	2.632
7	Test	2	4	13.75	2.621
8	Test	2	4	13.25	2.584
9	Test	1	3	13.95	2.635
10	Test	2	4	15.15	2.718
11	Test	1	3	13.15	2.576
12	Test	2	4	21.0	3.045
13	Test	1	3	8.75	2.169
14	Test	2	4	17.35	2.854
15	Test	2	4	18.25	2.904
16	Test	1	3	19.05	2.947
17	Test	2	4	15.1	2.715
1	Reference	1	2	13.5	2.603
2	Reference	1	2	15.45	2.738
3	Reference	1	2	11.85	2.472
4	Reference	2	1	13.3	2.588
5	Reference	1	2	13.55	2.606
6	Reference	2	1	14.15	2.650
7	Reference	2	1	10.45	2.347
8	Reference	2	1	11.5	2.442
9	Reference	1	2	13.5	2.603

(Continued)

Table 3 Results of a Four-Period, Two-Sequence, Two-Treatment, Replicate Design ($C_{\max}$) (Continued)

Subject	Treatment	Sequence	Period	$C_{\max}$	$\text{Log}_e(C_{\max})$
10	Reference	2	1	15.25	2.725
11	Reference	1	2	11.75	2.464
12	Reference	2	1	23.2	3.144
13	Reference	1	2	7.95	2.073
14	Reference	2	1	17.45	2.859
15	Reference	2	1	15.5	2.741
16	Reference	1	2	20.2	3.006
17	Reference	2	1	12.95	2.561
1	Reference	1	4	15.51	2.741
2	Reference	1	4	13.45	2.599
3	Reference	1	4	12.85	2.553
4	Reference	2	3	14.25	2.657
5	Reference	1	4	12.55	2.530
6	Reference	2	3	15.45	2.738
7	Reference	2	3	9.85	2.287
8	Reference	2	3	12.35	2.514
9	Reference	1	4	10.98	2.396
10	Reference	2	3	14.23	2.655
11	Reference	1	4	13.65	2.614
12	Reference	2	3	22.9	3.131
13	Reference	1	4	6.87	1.927
14	Reference	2	3	16.21	2.786
15	Reference	2	3	16.55	2.806
16	Reference	1	4	19.95	2.993
17	Reference	2	3	12.54	2.529

were changed and, in fact the probability of success has been reduced from 0.500 to 0.375.

How does an interim look affect the probability of getting a bad product to market (i.e., consumer risk)? This probability is also reduced. The two, one-sided t -test procedure has a maximum probability of 0.050 of getting a nonbioequivalent product approved. This means that, at most, 5% of the time a study on a nonbioequivalent product will have a 90% CI that falls within the BE limits, 80% to 125%. Some portion of these 5% of passing studies will look good at the interim evaluation and will thus be continued to an erroneous end. This portion will be unaffected by the interim analysis. The remaining portion of these studies will look bad at the interim look and will be terminated, even though had they been completed, the nonbioequivalent product would have produced bioequivalent results. It is clear that studies incorporating an interim look will get nonbioequivalent products approved less than 5% of the time.

Table 4 Analysis of Log_e(C_{max}) for Average BE

The MIXED Procedure																			
Class Level Information																			
Class	Levels	Values																	
SEQ	2	1 2																	
SUBJ	17	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17																	
PER	4	1 2 3 4																	
TRT	2	A B																	
REML Estimation Iteration History:																			
Iteration	Evaluations	Objective										Criterion							
0	1	−100.0397288																	
1	2	−170.0162313										0.00000045							
2	1	−170.0162698										0.00000000							
Convergence criteria met.																			
Covariance Parameter Estimates (REML):																			
Cov Parm	Subject	Group										Estimate							
FA(1,1)	SUBJ											0.19784008							
FA(2,1)	SUBJ											0.23184930							
FA(2,2)	SUBJ											0.09116812							
DIAG	SUBJ	TRT A										0.00809499							
DIAG	SUBJ	TRT B										0.00536364							
Tests of Fixed Effects:																			
Source	NDF	DDF										Type III F					Pr > F		
SEQ	1	15										0.96					0.3433		
PER	3	32.9										0.33					0.8061		
TRT	1	15										10.15					0.0061		
ESTIMATE Statement Results																			
Parameter	Estimate	Std Error										DF	t	Pr> t	Alpha	Lower	Upper		
T VS. R	0.09849653	0.03092097										15	3.19	0.0061	0.1	0.0443	0.1527		
Least Squares Means																			
Effect	TRT	LSMEAN										Std Error					DF	t	Pr > t
TRT	A	2.71471181										0.05049062					15	53.77	0.0001
TRT	B	2.61621528										0.06182155					15	42.32	0.0001

Note: 90% CI = Exp(Lower) to Exp (Upper) = Exp(0.0443) to Exp(0.1527) = 1.045 to 1.165, which is within the 0.800 to 1.250 FDA criteria, indicating that the Test (A) is bioequivalent to the Reference (B).

Reducing the probability of putting a good product on the market increases the producer risk. Reducing the probability of putting a bad product on the market decreases the consumer risk. While regulatory agencies such as the FDA desire to approve good products, it can be argued that it is more important that they keep bad products from getting to the marketplace. Because the interim look reduces the consumer risk, such a practice should not be of concern to the regulatory agencies.

Group Sequential Design

The group sequential design is when additional subjects can be dosed when the results from a first group of subjects does not meet some criteria. This type of study requires proper methods of analysis which can be derived from group sequential methods (early stopping rules) used in clinical trials. When our interest is only to stop early based on a successful outcome (i.e., demonstrating BE), the methods of Pocock, O'Brien and Fleming (9) or, the more general Wang and Tsatis' approach (9), can be adapted for use.

The use of Pocock's approach is illustrated by the two-stage, sequential, crossover, BE studies involved in the approval of Nexium Delayed-Release Capsules (10) in the United States. The active ingredient in Nexium is esomeprazole sodium, the sodium salt of the S-enantiomer of omeprazole, a potent inhibitor of gastric acid secretion. In each BE study, an initial group of 36 subjects was dosed and if the 94% CI for the geometric mean Test-to-Reference ratios for AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were contained within the interval 80% to 125%, then the study was stopped as a success. Otherwise, an additional group of 36 subjects was dosed and the 94% CI criteria was applied to the combined data ($N = 72$) from the two dosing groups. Had the study been conducted on a single group of 72 subjects, the traditional 90% CI would have been applied, rather than the 94% one used in the two-stage design. In the two-stage design, the use of a 94% CI is equivalent to performing each of the two, one-sided t -tests at a level of significance equal to 0.03 rather than at the conventional 0.05 level. [Note that $100\% * (1 - (2 \times 0.05)) = 90\%$ and $100\% * (1 - (2 \times 0.03)) = 94\%$.]

This same sequential approach, based on Pocock's test, can be extended to additional equally-sized dosing groups. Had the Nexium studies been conducted in three sequential groups, each with 24 subjects ($N = 72$ total if carried to completion), the results of the first group would have been evaluated to determine if a second group was needed. If the second group was required, then the combined results of the first two dosing groups would have been evaluated before proceeding, if needed, to the third group. The difference between this three-stage approach and the two-stage one is that the CI level at each analysis in the three-stage design needs to be 95.4% rather than the 94% level.

Add-on Design

Another type of group sequential design is that commonly referred to as the Add-on. In Canada, if a study fails because it was not sized sufficiently, an additional number of subjects may be studied so that the combined, total number of subjects would be sufficient to pass the study based on results of the initial failing study. This reduces the cost to the pharmaceutical company, which, otherwise, would have to repeat the entire study with a larger number of subjects. It is not a requirement that each group separately pass the CI requirement. Besides evaluating for a group-by-treatment interaction in the statistical analyses of the combined data set, a second statistical test is required when an Add-on group is involved. Each group is analyzed separately in the usual manner. The residual variances from the two separate groups are compared using an F test. If the variances are significantly different, the groups cannot be pooled and the product will probably fail. Note that the second group is studied only if the original study failed because of lack of size. It is possible that the Add-on study could pass on its own, and in this case, the Test product would be acceptable. This second test comparing variances seems rather onerous, because an analysis is possible for the combined groups with unequal variance. However, it may be the intention of the Canadian TPD to trade the benefit of the add-on design for unnecessarily more stringent regulatory requirements. The difficulty with determining the valid statistical method for this type of study is that the conditions which would trigger the Add-on group and what the size of that group will be in relationship to the originally dosed group are not often pre-specified in the protocol. With formal pre-specified criteria concerning the use of an Add-on group and under what conditions the study will be stopped as a success or as a failure without dosing an Add-on group, some adaptation of the Inner Wedge test (9) used for early stopping in clinical trials may provide an appropriate statistical approach. Until such time, the use of the Add-on group is a statistically controversial approach.

Adaptive Designs

Adaptive designs (11) encompass a number of innovative approaches to clinical research. These include the embedded pilot study, where a small group of subjects are dosed, their results evaluated to determine a sufficient size for the study and then after reaching this size, all data are combined and analyzed together. This can also include an interim evaluation to determine if the pre-specified size of a study is appropriate with regards to the accumulating data, and, if it is not, the adaptive methods allow for sample size adjustment in midstream. These approaches could be very important and cost-effective in BE testing, especially for products that are highly variable, where pilot studies often underestimate or overestimate the true within subject variability.

An intensive study of the appropriateness and properties of adaptive methods is being investigated by FDA and industry personnel in the United States at the time of this writing. A final finding will hopefully, be forthcoming in the near future.

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Population Pharmacokinetic Approaches for Assessing Bioequivalence

Philippe Colucci

Faculté de Pharmacie, University of Montreal, Montreal, Quebec, Canada

Jean-Francois Marier

Pharsight Corporation, Mountain View, California, U.S.A.

Murray P. Ducharme

*Cetero Research, Cary, North Carolina, U.S.A. and
Faculté de Pharmacie, University of Montreal, Montreal, Quebec, Canada*

INTRODUCTION

Population pharmacokinetic (PK) approaches were first introduced in the 1970s mainly as a tool to allow extraction of meaningful and useful PK data from sparse observational data arising from diverse clinical studies (1). It was quickly taken up by academicians and used to determine the PK and pharmacokinetics/pharmacodynamics (PK/PD) of drugs given to patients with more robustness than individual type approaches. Indeed, it was rapidly shown that population approaches were likely better than the standard two-stage approach for determining the PK of a compound from simulated data sets (2,3).

An important shift toward the use of population approaches in the drug development process of new drugs began in the 1990s. While regulatory submissions rarely included population PK approaches in the 1980s, nowadays it is estimated that virtually every new drug application to the Food and Drug Administration (FDA) includes a population PK component in the submission. In addition, regulators are more and more concerned

about optimizing the drug development process, as the FDA emphasized with its recent critical path initiative (4), and one way of doing this is by using more sophisticated modeling and simulation approaches.

So while the industry and regulatory bodies have embraced the use of population PK approaches for the development of new drugs, its use for the assessment of bioequivalence (BE) is still in its infancy.

COMPARTMENTAL VERSUS NONCOMPARTMENTAL PK APPROACHES FOR BE

As seen in previous chapters, BE studies examine whether two different formulations of the same drug behave similarly in terms of their rate and extent of exposure/bioavailability. Assuming that both drug formulations arrive in the systemic circulation before moving to their sites of efficacy and toxicity, if their systemic concentration-time profiles are identical, then the efficacy and toxicity resulting from this same exposure will also be identical.

Two formulations of the same drug will therefore be bioequivalent if they produce identical systemic concentration-time profiles, understanding that once the drug itself reaches the systemic circulation, its pharmacokinetic behavior (distribution and elimination) will be exactly the same between the two formulations. PK studies can be based on either compartmental or noncompartmental approaches.

The Compartmental PK Approach

This is the classical pharmacokinetic approach where observed concentrations are fitted mathematically and statistically to a model comprised of compartments.

The compartmental PK approach involves fitting the experimentally observed concentrations using a mathematical model. Although used as a reference approach in PK, its use in BE is limited for reasons that we will elaborate upon in the following section.

The rate and extent of exposure/bioavailability of two different formulations of the same drug are compared by examining the pharmacokinetic parameters “ k_a ” (absorption rate constant) and F_{rel} (relative bioavailability between the two formulations). All other PK parameters (volumes of distribution and clearances) should be identical for both formulations, as once the drug reaches the systemic circulation, it will behave similarly regardless of formulation performance. In the simplest case and as presented in [Figure 1](#), BE comparison between two formulations of the same active ingredient will therefore be implemented by fitting data to a model incorporating two absorption rate constants (e.g., one for the test and one for the reference formulations), a relative bioavailability factor (F_{rel}) assessed when the data from the test formulation is being fitted, and all “systemic” PK parameters (e.g., volumes of distributions and clearances) will be fitted to be the same

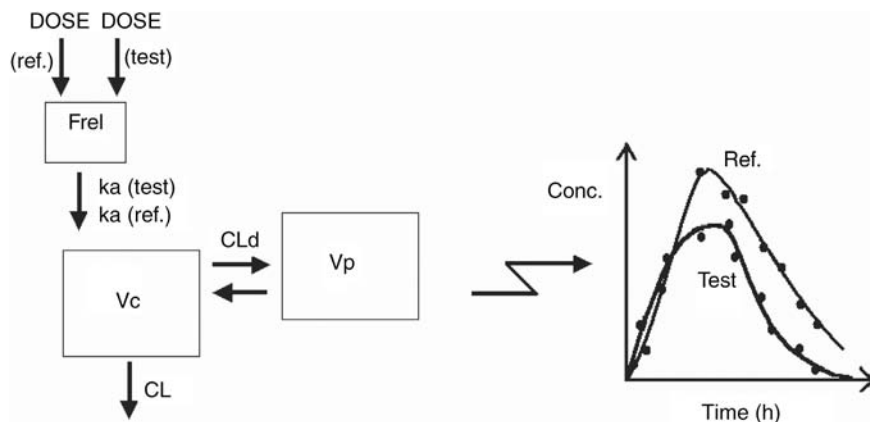


Figure 1 Extravascular drug administration–two compartment model. *Abbreviations:* CL, Apparent total body clearance; CLd, apparent distributional clearance; Conc, concentrations; F_{rel} , relative bioavailability between the test and reference (ref.) formulations; k_a , absorption rate constant; V_c , apparent central volume of distribution; V_p , apparent peripheral volume of distribution

between the two formulations. In essence, these apparent volumes of distribution and clearances will be fitted as CL/F_{rel} and V/F_{rel} , where F_{rel} will be fixed to a value of 1 for the reference product, while it will be calculated for the test product. This F_{rel} parameter will be the population ratio representing the relative bioavailability of the test over the reference product. Should study data be amenable to be calculating with both the noncompartmental and the compartmental approach, the results of the ratio of AUC_{inf} (test over reference) with the first approach will be the same as the F_{rel} obtained with the latter approach. Should absorption lag times be needed for the data to be properly characterized, then these parameters will be allowed to be different from one period to the other and between formulations.

Although compartmental pharmacokinetics can be considered as the gold standard approach in many phases of drug development (e.g., in sparse sampling situation and in PK/PD studies) and clinical practice (e.g., by pharmacists and physicians), it is not favored in BE for a multiple of reasons. First, compartmental PK is a complicated modeling approach demanding experienced modelers. Second, because compartmental PK involves data fitting, the results are highly prone to experimental uncertainty, making it extremely difficult to prove that two formulations are bioequivalent even when they are. For these reasons, the compartmental approach in BE should therefore be reserved for situations where the noncompartmental PK cannot be used (see below), or when additional information that noncompartmental PK cannot provide is useful and necessary.

The Noncompartmental PK Approach

This approach is based on the calculation of an area under the curve (AUC), which represents the body's exposure to the drug. Three PK parameters are important to consider to indicate that two formulations of the same active ingredient are bioequivalent: The maximum observed drug concentration ($C_{\max}$), the observed exposure (AUC from time zero to the last detectable concentration, AUC_{0-t}), and the complete exposure (the AUC extrapolated to infinity, AUC_{inf}).

The noncompartmental approach involves relatively simple calculations such as AUC and $C_{\max}$. It is the preferred method for BE assessment because of its simplicity and robustness in rich sampling studies (Fig. 2).

The AUC_{0-t} and AUC_{inf} parameters reflect the overall extent of absorption while the $C_{\max}$ parameter reflects both the rate and extent of exposure/bioavailability.

The noncompartmental PK is the usual method favored to prove BE because of its robustness. The experimental uncertainty (e.g., due to analytical assay variability, experimental errors, variability in the administered dose, etc) associated with each drug concentration time point does not contribute to the variability of the overall AUC. This is because of the numerous concentration points obtained in a rich sampling study (a minimum of 15 samples after a single dose administration), and the fact that a rich sampling schedule ensures that the uncertainty associated with each time point cancels itself out (5). Rich sampling schedules associated with typical BE studies require a minimum of 15 samples after a single dose administration to calculate the AUC. If the nature of the study necessitates a smaller sampling schedule, as in pediatric or BE studies performed in patients (e.g., in oncology), then the compartmental PK approach can be very useful as we will see in this chapter.

THE POPULATION PK APPROACH

When the PK of a drug needs to be analyzed using compartmental methods, the population approach can be very useful. As a rule of thumb, it is always

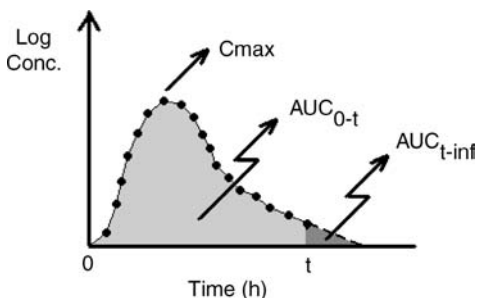


Figure 2 The noncompartmental PK approach. *Abbreviations:* PK, pharmacokinetic; AUC, area under the curve; $C_{\max}$, maximum observed drug concentration.

better to perform compartmental analyses using a population approach (2,6–8). At best, the results will be much more robust and at worse, they will be similar to those observed using individual compartmental methods.

Population PK Methods

The pharmacokinetic behavior of a drug can be assessed using population PK approaches that range from simple to complex.

Consider a simple case (Table 1) involving a population of three subjects taking an investigational drug in a hospitalized setting and where the total clearance (CL) and the total volume of distribution (Vss) are to be calculated:

The Pooled Data Approach

In this approach, all of the data are pooled together as if all subjects would be different (Table 2). Sheiner has deemed this approach “naïve” because inter- and intra-individual variabilities are not determined (2). The population PK results would therefore be calculated as if the PK behavior of the drug would be known in four different subjects instead of three.

The Standard Two-stage Approach

The standard two-stage approach overcomes the “naiveness” of the pooled approach by first calculating the PK behavior of a drug in each subject and on every occasion that the subject receives the drug (Table 3). This is the first stage of the method. In the second stage, the population PK results are calculated in a standard fashion.

It can be seen that the results are different compared to the naïve pooled data approach, where because subject No. 1 was counted twice, the mean and variability data were biased.

Mixed-Effect Modeling Approaches

Mixed-effect modeling approaches are much more complicated than the preceding ones since they include what are called fixed effects, random effects and the use of individual specific information called covariates (1,2,7). Fixed effects are the population fitted parameters while the random

Table 1 Individual PK Parameters from Three Subjects Taking an Investigational Drug

Patient number	CL (L/h)	Vss (L)
1 (May 2005)	1	24
1 (September 2005)	0.8	24
2	1.5	18
3	1.6	17

Abbreviations: CL, total clearance; Vss, total volume of distribution.

Table 2 Population PK Parameters Using the Pooled Data Approach in the Three Subjects Taking an Investigational Drug

Patient number	CL (L/h)	Vss (L)
1 (May 2005)	Pooled	Pooled
1 (September 2005)		
2		
3		
Population mean	1.2	20.8

Abbreviations: CL, total clearance; Vss, total volume of distribution.

effects refer to the inter-subject variability in the different fitted PK parameters and the residual variability (often termed intra-subject variability) in the observations that are being fitted or explained. Consider two main approaches, the first method known as the NONMEM® process, which is regarded as the gold standard, and the other approach, the Iterative two-stage method. Many other approaches are now available, including some nonparametric procedures but they will not be presented here.

NONMEM®: This mixed-effect modeling approach was first introduced by Beal and Sheiner and, the approach was implemented by a software package called NONMEM® (9,10). This approach models the data in one stage and calculates the population average parameters and the inter-subject variability as well as the residual variability in the observations. Should the individual parameters be requested, then a *posthoc* analysis using a Bayesian approach can be performed in a second stage.

This method has consistently performed well compared to other proposed approaches, and in particular, has always been proven to be either similar or superior to the standard two-stage approach (2,6–8,11). It is also the method of choice when it is necessary to see if covariates can help

Table 3 Population PK Parameters Using the Standard Two-Stage Approach in the Three Subjects Taking an Investigational Drug

Patient number	CL (L/h)	Vss (L)
1 (May 2005)	1	24
1 (September 2005)	0.8	24
1 (average results)	0.9	24
2	1.5	18
3	1.6	17
Population Mean	1.33	19.7
InterCV	28.4%	19.3%

Abbreviations: CL, total clearance; Vss, total volume of distribution.

explain the pharmacokinetic behavior of a drug. In NONMEM[®], covariates are easily added whether they are continuous or binomial in nature, and their inclusion in the model is a relatively simple and robust procedure.

The Iterative Two-Stage Approach (IT2S[®]): This method is derived from the work of Prevost and Steimer, and has been implemented by Collins and Forrest in a software package called IT2S[®] (3,12,13). It is a mixed-effect modeling approach that allows, similar to NONMEM[®], the modeling of observational data and reporting of population averages, inter-individual variability and residual variabilities in fitted data as well as individual parameters. The iterative two-stage approach derives its results in an opposite manner to NONMEM[®] since it first computes the individual PK parameters and then calculates population PK parameters in successive iterations until convergence is achieved. IT2S[®] has been used extensively to provide more robust estimations of individual pharmacokinetic parameters compared to using a standard two-stage approach (14–18). The iterative two-stage approach has been compared and shown to be superior to the standard two-stage approach (11,13), but to our knowledge has not been directly compared to the NONMEM[®] approach. One advantage of the iterative two-stage approach over NONMEM[®] is that it uses ADAPT-II[®] subroutines (19), and there is no limitation on the complexity of the model, the number of parameters to be calculated or the number of observations to be fitted. The disadvantages compared to NONMEM[®] are that it is computationally slower and it is not well suited for the testing of covariates that are other than continuous in nature.

Comparison of Population versus Individual Compartmental Approaches

As previously mentioned, the mixed-effect modeling approaches, NONMEM[®] and IT2S[®], have each been compared to the standard two-stage approach and shown to be vastly superior in distinguishing between the true inter-individual variability in PK parameters and the uncertainty associated with the observational data to be fitted (e.g., the residual variability). Advantages and disadvantages of the different methods are presented in [Table 4](#).

POTENTIAL AND/OR ADVANTAGES OF POP PK APPROACH FOR THE ASSESSMENT OF BE

The gold standard for pharmacokinetic analysis of BE studies has been the noncompartmental approach for several years now. The reasons are self-evident; the methodology is sound, simple and robust when a sufficient number of blood samples are available. Because it does not use data fitting, the results are not dependent on specific modelling experience of the scientist. There are, however, some situations where it will not be

Table 4 Comparison of Different Population PK Methods

Analysis method	Advantage	Disadvantage
Naïve pooled data	Simple and easy to perform	Not accurate Inter- and intra-individual variability combined
Standard two-stage	Simple and easy to perform Point estimates are typically reasonably well estimated	Typically overestimates inter variability in PK parameters Does not discriminate well between inter- and intra-individual variability Requires a large number of observations per subjects
NONMEM®	Can be used in sparse and rich sampling situations Discriminates between inter- and intra- individual variability Robust evaluation of population and individual PK parameters Evaluation of covariates	Time and computationally intensive Complex analysis Limits on the complexity of the model
IT2S®	Can be used in sparse and rich sampling situations Discriminates between inter- and intra- individual variability Robust evaluation of population and individual PK parameters No limitation on the complexity of the model	Time and computationally intensive Complex analysis Limited evaluations of covariates

Abbreviation: PK, pharmacokinetic.

scientifically correct or appropriate to use the noncompartmental approach. For example, when the pre-requisites for this approach are not met, such as sparse blood sampling in patients, when the pharmacokinetic behavior of the drug is nonlinear, or when plasma data are not available due to low bioavailability of the drug or analytical limitations. Furthermore, the compartmental approach is likely to be more restrictive than the non-compartmental approach since it compares the absorption rate constants of two different formulations rather than the maximum drug concentration obtained from a single observation. It is therefore much more difficult to

actually prove BE between two formulations using a compartmental approach than with a noncompartmental method.

Consider the following case examples where some of the limitations of the noncompartmental approach are involved.

CASE STUDIES

BE Assessment When Only Urinary Data Are Available

Example is alendronate.

Background Information on Alendronate

Alendronate sodium, an aminobisphosphonate drug, inhibits bone resorption by binding to bone hydroxyapatite leading to progressive gains in bone mass (20). Alendronate is indicated for the treatment and prevention of osteoporosis, glucocorticoid-induced osteoporosis and for the treatment of Paget's disease of bone. The recommended doses of alendronate sodium are 10 mg daily or 70 mg once weekly for osteoporosis and 40 mg daily for Paget's disease (21).

The oral bioavailability of alendronate is approximately 0.6% under fasting conditions (21). Following a single IV dose of [^{14}C] alendronate, 50% of the radioactivity is excreted in urine over 72 hours, with little or none recovered in the faeces (20,22). The remainder of the available dose is thought to bind to bone, and is therefore expected to be excreted along with bone resorption over a period that can last to up to 10 years (22). Alendronate appears to display dose-dependent linear pharmacokinetic behaviour, as the percentage of an oral dose excreted in the urine over 36 hours appears to be similar when doses are administered between 5 and 80 mg (23).

Although alendronate has been marketed for several years, it has not been yet possible to measure its plasma or serum concentrations after oral administration because of its low bioavailability and analytical limitations. Analytical methods are only available to assay urinary excreted amounts of the drug. As in similar situations when systemic drug concentrations cannot be obtained, or when the drug concentrations do not relate to the efficacy/toxicity of the drug, clinical studies have to be performed to determine whether or not two formulations of the same active ingredient are bioequivalent. To reflect this, communications available from the office of generic drugs (OGD) of the FDA in early 1999 suggested that pharmaceutical companies interested in developing a generic formulation of alendronate need to perform a clinical study using a bone marker to show that the clinical activity of the proposed test formulation would be similar to the marketed reference product (24).

Although such a study design would have clearly demonstrated whether or not a generic formulation of alendronate displayed similar pharmacological activity as the reference formulation, multiple generic companies judged this approach not feasible for various reasons such as the extremely large number of subjects required for the analysis and a very long follow-up including a high financial implication.

Methodology

It is possible, however, to prove that two different formulations of alendronate have similar rate and extent of exposure/bioavailability following population compartmental modeling of urinary data only. In an analogous manner to plasma data, it is feasible to determine both the absorption and the elimination phases of alendronate from urinary data because this drug exhibits the following pharmacokinetic characteristics: once absorbed, alendronate is not metabolized, is only excreted unchanged in the urine and it is not eliminated in the bile (20,22). Because of this, the bioavailability of alendronate can be estimated from the amount of drug excreted unchanged in the urine. The total amount of alendronate recovered in urine up to infinity will therefore correspond to the absorbed and bioavailable dose. As previously mentioned, the only difference that can exist between two formulations of the same active ingredient resides in the respective rates and extent of exposure/bioavailability. The extent of exposure of alendronate is therefore described by calculating the bioavailability of the test and reference formulations (i.e., F_{test} and $F_{\text{reference}}$) whereas the rate of absorption is described by their respective k_a 's (i.e., $k_{a\text{test}}$ and $k_{a\text{reference}}$). The time course of amounts excreted in urine and the urinary excretion rate at specific collection intervals will be indicative of both the absorption rate constant (k_a) and of the elimination rate constant (k_{el}) of alendronate. Since k_{el} should be constant within subjects and between formulations containing the same active, the time course of alendronate excreted in urine over specific collection intervals can be simultaneously fitted to estimate one k_{el} and two k_a 's (i.e., one for each alendronate formulation). Because of these characteristics, the amount of alendronate excreted in urine over multiple collection intervals (i.e., over a 36-hour collection period) can be used instead of systemic drug concentrations to provide an indication on how much and how fast the drug reaches the systemic circulation.

The current alendronate studies were designed as two-way crossover trials where healthy subjects received single oral doses of alendronate of the test (generic) and reference (innovator) products in a randomized manner to assess BE between the two products. A total of 10 urinary samples were collected over multiple intervals such as: -1 to 0.5, 0.5 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 24 and 24 to 36 hours. The early collection intervals (i.e., first 2 to 3) are crucial for an accurate determination of the absorption rate of the drug. It is therefore recommended to administer

sufficient volumes of water to the subjects to make sure they are able to void urine at the end of these early intervals.

Noncompartmental Pharmacokinetic Analysis: The total amount of alendronate excreted unchanged in urine over an entire 36-hour period of sample collection (A_{e0-36}) can be obtained by adding the amounts excreted over each collection interval. The urinary rate of excretion (R) of each collection interval can be calculated by dividing the amount of drug excreted by the time over which it was collected. The $R_{\max}$ corresponds to the maximum rate of excretion of alendronate and $T_{\max}$ the midpoint of the corresponding interval.

Population Compartmental Pharmacokinetic Analysis: Pharmacokinetic analyses were performed using standard compartmental methods (25). The simplest model that best fitted the urinary excreted amounts of alendronate was a one compartment PK model with first-order absorption and elimination. The schematic representation of this model is shown in Figure 3.

Parameters defined by the model included two bioavailability factors (F_T and F_R for the Test and Reference formulations, respectively), two first-order absorption rate constants (ka_T and ka_R for the Test and Reference formulations, respectively), one first-order elimination rate constant (kel), and two lag times ($Tlag_T$ and $Tlag_R$ for the Test and Reference formulations, respectively). The lag times of absorption represent the amount of time necessary before the absorption of the drug begins. These parameters had to be included in the compartmental analysis to get robust estimates of ka and were allowed to change between and within individuals. Individual

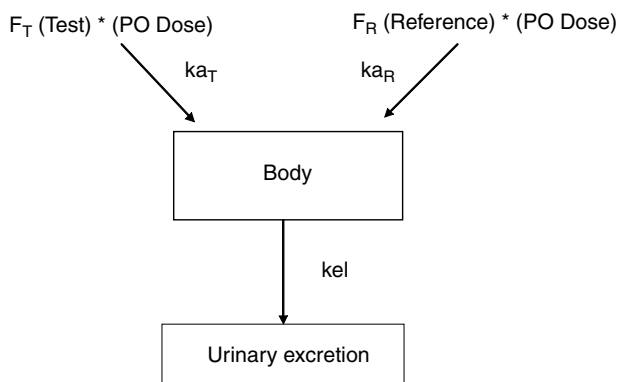


Figure 3 Compartmental model of excreted urinary amounts of alendronate using a population PK analysis. *Abbreviation:* PK, pharmacokinetic.

pharmacokinetic parameters were derived using maximum likelihood analysis with ADAPT-II® (19). The median of the individual PK parameter estimates obtained from ADAPT-II® were then used as prior values for a population analysis performed with the iterative two-stage methodology (IT2S®) (12). This method was used to estimate more robustly the individual pharmacokinetic parameters rather than to estimate the population parameters. All urinary amounts of alendronate recovered in urine were modeled using a weighting procedure of $W_j = 1/S_j^2$ where the variance S_j^2 was calculated for each observation using the equation $S_j^2 = (a + b*Y)^2$. The parameters a and b are the intercept and slope of the variance model. The slope is the proportional component of the residual variability associated with each urinary amount (sum of the intra-individual variability and the sum of all experimental errors), while the intercept is the additive component.

Statistical Analysis: Most regulatory guidelines usually require the calculation of point estimates of key BE PK parameters as well as the 90% confidence intervals (CIs) to be between 80-125% (e.g., FDA Bioavailability and BE guideline) (26). Consistent with the two one-sided test for BE (27), 90% CIs for the difference between drug formulation least-squares means (LSM) were calculated for the ln transformed noncompartmental (Ae_{0-36} and R_{max}) and compartmental (k_a and F) PK parameters. The statistical analyses were performed using the SAS® GLM procedure. All ratios and 90% CIs were expressed as a percentage relative to the reference formulation.

Results and Discussion

As previously described, the total amount of alendronate recovered in urine is representative of the bioavailable dose and can be estimated with robustness, whether by compartmental (F) or noncompartmental analyses (Ae_{0-36}) because of the sampling strategy (10 observations per patient per treatment, which is rich for urinary data). A comparison of the results from these two approaches is therefore relevant, as it will indicate whether ratios and CIs from the compartmental analysis are consistent and as robust as those observed with the noncompartmental analysis. Average results from various studies performed at MDS Pharma Services are presented in Table 5.

Based on the above results, the PK parameters representing the extent of exposure/bioavailability estimated by compartmental analyses were similar to those observed when using noncompartmental analyses, thereby confirming the consistency of the two analyses.

Compared to the compartmental analysis, which allowed the calculation of the rate of exposure/bioavailability (k_a) of alendronate from the two formulations, the current noncompartmental analysis did not provide any direct measurements of this parameter. The closest parameter providing information on the rate of exposure/bioavailability of the drug using

Table 5 Pharmacokinetic Parameters Estimated Using Noncompartmental and Compartmental Methods from Five BE Studies of Alendronate

PK parameters		Ratio of LSM (90% CI)	
		Noncompartmental PK analysis	Population compartmental PK analysis
Extent of exposure/bioavailability	F	N/A	101.4 (94.4–108.9)
	Ae_{0-36}	99.2 (89.8–109.5)	N/A
Rate of exposure/bioavailability	ka	N/A	94.0 (89.6–98.6)
Other (maximum rate of elimination)	R_{max}	99.4 (90.8–108.8)	N/A

Abbreviations: Ae_{0-36} , amount excreted in urine from time zero to 36 hours; F , bioavailability; ka , absorption rate constant; LSM, least-squares means; N/A, not applicable; PK, pharmacokinetic.

noncompartmental method is the maximum rate of excretion ($R_{\max}$). Theoretically, if one would be able to collect a minimum of five different urinary samples likely to provide information on both the absorption and the elimination of the drug, then the parameter $R_{\max}$ would almost coincidentally be seen with that of the maximum plasma concentration, because the maximum amount of drug eliminated in the urine will happen immediately at or after systemic concentrations of the drug are maximum. Because we are dealing with collected urinary amounts, however, the maximum number of collectable urinary samples within the first 2 hours of sampling after dosing is three. Because of this sparse sampling situation, the $R_{\max}$ may not provide robust information to discriminate adequately the rate of exposure/bioavailability of two different formulations of alendronate. With the compartmental analysis, however, the amounts of alendronate excreted in urine for the two formulations are fitted simultaneously. Within the collected urinary samples, information is available on the rates describing both the absorption and the elimination of the drug, and because the elimination of alendronate between the two formulations is the same, this method allows one to robustly discriminate the rate of exposure/bioavailability of two different formulations of alendronate.

All point estimates and 90% CIs for PK parameters were within the acceptable range for BE of 80% to 125%. Following the submission of study reports using the approach described here, the OGD of the FDA sent a letter to the industry stating that they would accept BE studies for alendronate using urinary data instead of doing a study with clinical endpoints (28).

Conclusion

The one-compartment PK model with first-order absorption and elimination well-described, the amounts of alendronate excreted in urine for both formulations. The population approach was shown to be a useful tool to determine whether two different formulations of alendronate have similar rate and extent of exposure/bioavailability.

BE Assessment When Only Efficacy Data Are Available

Example is albuterol.

Background Information on Albuterol

Albuterol is an adrenergic β_2 -agonist widely used in the treatment of reversible airways obstruction in asthmatic patients. To minimize side effects and promote local activity, this drug is given through inhalation (29,30). Because of the recent international restriction concerning the use of chlorofluorocarbons (CFCs) as a system propellant, over the last 10 years, pharmaceutical companies had to develop alternative systems of aerosolized

drug delivery (31,32), and demonstrate that new formulations were bio-equivalent to the old CFC products. As previously mentioned, a fundamental assumption ensuring that two formulations containing the same active ingredient will result in the same activity and toxicity is that the drug must first be absorbed into the systemic circulation and then distribute to the different sites of activity/toxicity.

Many consider that this assumption is not met for this compound, and consider that albuterol acts “topically” when administered by inhalation. Because of this, the FDA proposed that the most practical method of showing therapeutic equivalence between two formulations of albuterol was through the use of PD measures (33). The $E_{\max}$ dose-scale method is one of the currently accepted method to assess the dose-response of albuterol after multiple doses (34–37). The shape of the dose-response curve of albuterol is particularly important because marked differences in drug potency between two formulations may result in a small change in response, especially if the dose administered is too high. The $E_{\max}$ dose-scale method is associated with multiple assumptions which contribute to a high degree of uncertainty. This typically results in an overestimation of the CIs, which readily fall outside the 80% to 125% limit thereby requiring an unreasonably large number of patients to demonstrate BE (34–37).

This case study presents an alternative method to assess the BE of two different inhalation formulations of albuterol using PD data only. Instead of merely comparing the pharmacodynamic activity using forced expiratory volume in one second (FEV_1) between the two formulations using a dose-scale approach, the following considers the application of a PK/PD model in which the rate and extent of exposure/bioavailability of the two formulations can be robustly assessed using a population PK/PD analysis. The results of such an approach have previously been presented at a meeting of the American Society of Clinical Pharmacology & Therapeutics (38).

The data from a Phase II, randomized, double-blind, placebo-controlled, single and double dose, five-way crossover study in 60 mild to moderate asthmatic patients were used. The study involved delivering one or two actuations of albuterol sulfate from the test formulation and one or two actuations of albuterol from the reference formulation on four of the five study days. On one of the five study days, patients received placebo. Spirometry measurements (FEV_1) were taken at baseline and at 0.17, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 6 hours after the actuations.

Standard Dose-Scale Approach: As mentioned previously, an $E_{\max}$ dose-scale method is a typical method to demonstrate dose-response relationship of albuterol after multiple doses. This method was used as a comparison with the population approach described later in this case study. The $E_{\max}$ dose-scale model was based on the method of Gillespie et al. (19). The area under the effect curve from time 0 to 6 hours ($AUEC_{0-6}$) for FEV_1

data were calculated using the linear trapezoidal method for placebo and active treatments (single and double doses for the test and reference formulation). A dose-response profile was used to estimate the reference product dose that would produce a PD response equal to that resulting from the test product. The relationship between the actual dose of albuterol and the corresponding AUEC were fitted using the following equation:

$$\text{AUEC}_{(D)} = \text{AUEC}_{\text{Placebo}} + \text{AUEC}_{\text{max}} \times D / (\text{ED}_{50} + D),$$

where $\text{AUEC}_{(D)}$ is the observed AUEC at a given dose, $\text{AUEC}_{\text{Placebo}}$ is the AUEC after placebo administration, AUEC_{max} is the maximum theoretical increase in AUEC after administration of the active treatments, and ED_{50} is the effective dose of albuterol to achieve 50% of AUEC_{max} . A schematic representation of this model and the dose-scale approach is depicted in Figure 4 below.

The nonparametric 90% CI values were determined by bootstrapping the data from the original data set 500 times (39).

Population PK/PD Approach: A novel approach to determine the BE of these two formulations was also performed. As mentioned at the beginning of this chapter, the pharmacological response to a drug is always related to the concentration of the drug at the site(s) of activity. The only difference that can exist between two different inhalation formulations of albuterol is in their rate and extent of exposure/bioavailability, and not in

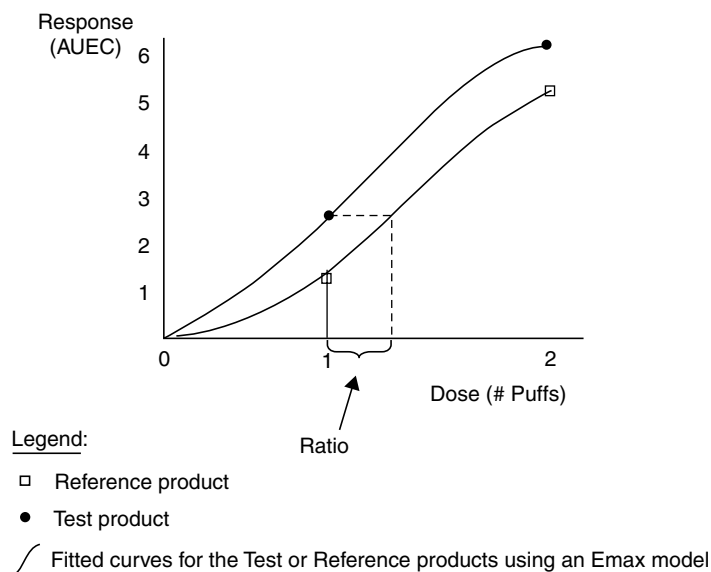


Figure 4 Schematic representation of the E_{max} dose-scale model. *Abbreviation:* AUEC.

the actual concentration-response curve of albuterol at the site of activity. From earlier investigations of the PK/PD of (R)-albuterol given by inhalation in dogs, we had observed that the pharmacological effect on heart rate was directly related to the concentrations observed in plasma. We also found that the absorption rate constant of the drug when given via inhalation was extremely rapid at about 20 per hour (40). In essence, it was found that when given by inhalation, the drug was absorbed extremely quickly and the pharmacological response of the drug was directly related to the drug's plasma concentrations. This is firstly due to the lung being extremely well perfused, so once a drug is absorbed through the lungs it appears almost immediately in the systemic circulation, and secondly the effect resulting from the binding to β_2 -receptors is directly related to the systemic concentrations observed.

In light of the foregoing, a PK/PD model was derived to provide discrimination between the rate and extent of exposure/bioavailability of two different formulations of albuterol using only FEV₁ observations. The model is presented in Figure 5 below.

A concentration-effect relationship was constructed for both the reference and test formulations described by the following formula:

$$FEV_{(t)} = FEV_{base} + E_{max} \times A/(EA_{50}/F + A),$$

where $FEV_{(t)}$ = the observed FEV₁ at any time points and FEV_{base} = the baseline FEV₁.

The maximum pharmacological response to albuterol (E_{max}) and the effective amount of albuterol to achieve 50% of E_{max} (EA_{50}/F) were influenced by the amount of albuterol (A) reaching the site of efficacy in the lungs (i.e., β_2 -receptor). Therefore, by modeling the appearance and disappearance of the bronchodilating effect of albuterol, one can deconstruct

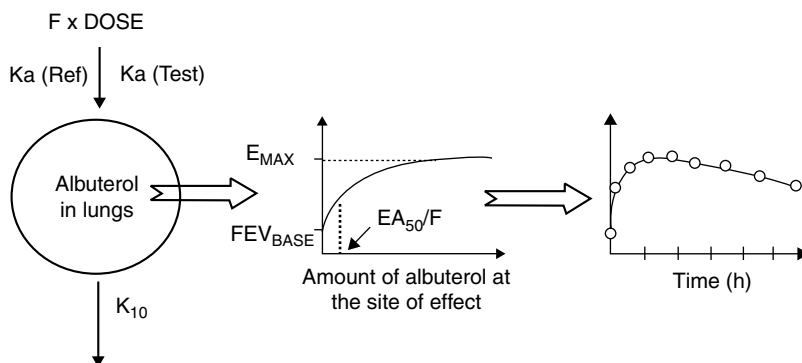


Figure 5 Schematic representation of the population PK/PD model. *Abbreviation:* PK/PD, pharmacokinetic/pharmacodynamic.

from this, the rise and elimination of albuterol systemic concentrations, and therefore the absence or presence of a difference in the rate and extent of exposure/bioavailability between the two formulations. This was done using the population analysis tools NONMEM[®] Version V and IT2S[®]. A one-compartment PK model was used to describe the pharmacokinetic behavior of albuterol using first order rate constants of absorption [k_a (test) and k_a (ref)] and elimination (k_{10}), and a relative difference in bioavailability (F_{rel}).

The population model was used to fit FEV₁ profiles after placebo administration, and after one and two actuations of albuterol in every subject. Population PK parameters, their inter-individual variability, and the overall residual variability were estimated using the First-Order Conditional Estimation method in NONMEM[®]. The point estimates (e.g., means) and their parametric 90% CIs were directly calculated (NONMEM[®]) or were calculated with SAS[®] GLM procedure using the individual results computed from IT2S[®].

Results and Discussion

Results for the conventional E_{max} dose-scale method are summarized in Table 6. The median values for the AUEC_{max} and ED₅₀ parameters were 2.99 L h/s and 49.5 μ g, respectively. The bootstrap analysis resulted in a median value for F_{rel} of 96% with CIs of 48 to 169%.

These results suggest that these two formulations of albuterol may be potentially bioequivalent because the point estimate is very close to 100%. However, the lack of precision of such a method results in a CI that is extremely wide at 48 to 169%, and the usual BE criteria of 80 to 125 is not met.

The results of the population analysis of FEV₁ data are summarized in Table 7. The point estimates and the 90% CI for $k_{a,rel}$ and F_{rel} were 108.0% (100.7% – 115.8%) and 97.7% (90.9% – 105.0%), respectively. The residual

Table 6 Modeling of AUEC Data Using an E_{max} Dose-Scale Model to Assess the BE Between Two Formulations of Albuterol

Parameters	Point estimates (90% CI) ^a FEV ₁
AUEC _{Placebo} (L h/s)	13.7 (12.7 – 14.7)
AUEC _{max} (L h/s)	2.99 (2.17 – 4.44)
ED ₅₀ ^{test} (mcg)	49.5 (15.9 – 96.8)
F_{rel}	0.96 (0.48 – 1.69)

^a Median and nonparametric 90% CI calculated by bootstrap.
Abbreviations: AUEC, area under the effect curve; AUEC_{max}, maximum increase in AUEC due to the active treatment above what can be seen with the placebo (AUEC_{Placebo}); AUEC_{Placebo}, AUEC observed after placebo administration; CI, confidence interval; ED50, effective dose that results in the attainment of 50% of the maximum increase in AUEC (AUEC_{max}).

Table 7 Population Modeling of FEV₁ Measurements Using an *E*_{max} Model to Assess the BE Between Two Formulations of Albuterol

Methods	Parameters	Ratio of LSM (90% CI)
NONMEM®	ka _{rel} (h ⁻¹)	1.08 (1.00–1.17)
	<i>F</i> _{rel}	0.97 (0.91–1.05)
IT2S®	ka _{rel} (h ⁻¹)	1.11 (1.01–1.21)
	<i>F</i> _{rel}	0.95 (0.89–1.01)

Abbreviations: CI, confidence interval; *F*_{rel}, relative difference in bioavailability between the test and the reference products; LSM, least-squares means; ka_{rel}, relative difference in the absorption rate constant between the test and the reference products.

variability left from the modeling of all of the FEV₁ data was 13.8%, indicating that the unknown or uncertainty added by the modeling of FEV₁ was either absent or at least kept to an absolute minimum by the model.

A graphical representation of the fit from IT2S® for a representative subject is presented in Figure 6, and the goodness of fit for the entire population of data with IT2S® is presented on Figure 7. Both NONMEM®

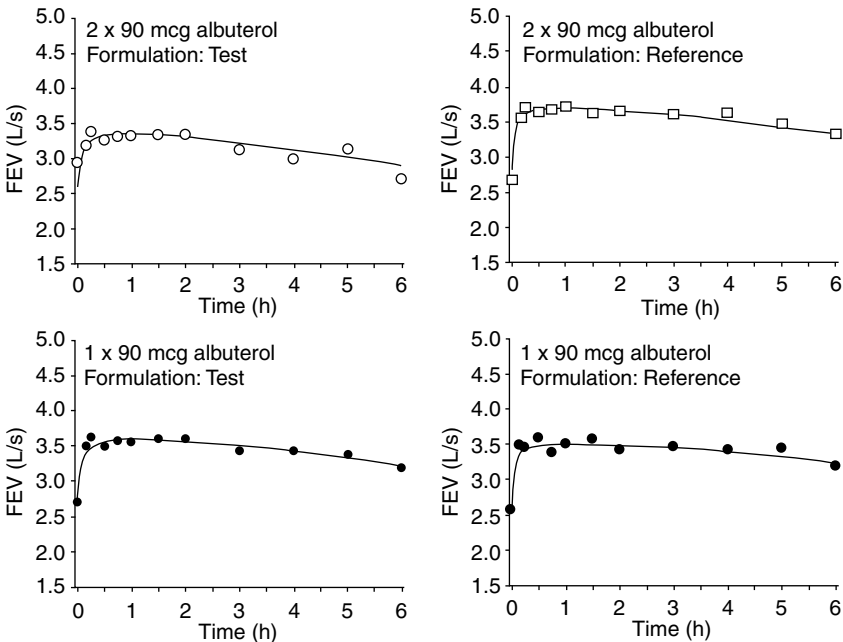


Figure 6 Observed (symbols) and fitted (lines) FEV₁ data in a representative subject using the population PK/PD model with IT2S®. Abbreviation: PK/PD, pharmacokinetic/pharmacodynamic.

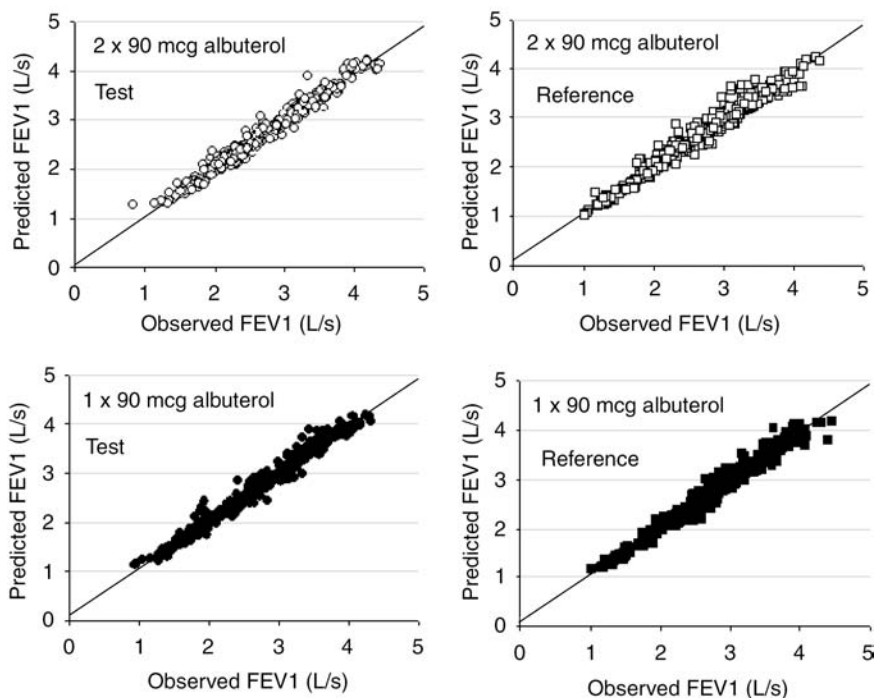


Figure 7 Observed and predicted FEV₁ data for the whole population of subjects using the population PK/PD model with IT2S[®]. *Abbreviation:* PK/PD, pharmacokinetic/pharmacodynamic.

and IT2S[®] resulted in similar excellent goodness of fit plots and so only one is presented here.

The dose-scale approach with bootstrap re-sampling (34,41) was used as a reference point to assess the BE between two formulations of albuterol. With this method, the median value for the parameter reflective of BE (i.e., F_{rel}) was within normal limits. However, the two formulations of albuterol were associated with 90% CI's falling outside of the generally accepted 80% to 125% limit by a large margin. We believe that this large CI is due to a limitation of the E_{max} dose-scale approach since only one parameter (F_{rel}) is allowed to explain all differences in dose-response curves between the two formulations. In addition, because the dose-scale approach uses only one observation (i.e., AUEC₀₋₆) per dose and per formulation, the BE is assessed only on 5 observations per patient (AUEC₀₋₆ for placebo, test1, test2, reference1, and reference2). This low number of observations may lead to uncertainty, resulting in overestimated CI's, even though the point estimates may be accurate.

Instead of assessing BE on only five observations per patients, individual FEV₁ profiles were modeled using all 48 observations per patient using a novel $E_{\max}$ PD model driven by a one-compartment PK model. Similar models have previously been used for the PD modeling of other drugs without drug concentrations (42–45). The parameters $E_{\max}$, EA_{50} and k_{10} are the same for both formulations, as they are albuterol-specific parameters. The only differences that can exist between these two formulations of albuterol are in their rate and extent of exposure, and these are captured by the relative differences in the absorption rate constants ($k_{a\text{rel}}$) and bioavailability (F_{rel}). This method dramatically decreased the uncertainty and resulted in a tighter estimation of the CI's. All CIs, calculated from NONMEM[®] or obtained via bootstrapping techniques were within the usual regulatory CI limits of 80% to 125%.

Conclusion

This case study described a novel PK-PD population model for the dose-response modeling of pharmacodynamic measures when drug concentrations are not available. This approach for albuterol can be used to assess the BE of two different formulations delivering the active compound to the lungs and was shown here to be advantageous compared to other methods used currently such as the dose-scale approach, because it resulted in more robust CIs for the BE parameters of interest. In addition, this model allowed a more standard evaluation of the BE between two formulations of albuterol, because it provides results in terms of rate and extent of exposure/bioavailability at the site of action.

BE Assessment in Patients

Example is cyclosporine.

Background Information on Cyclosporine

Cyclosporine (Neoral[®]) is a potent immunosuppressant medication. Cyclosporine inhibits Interleukin-2 activity and directly affects T-lymphocytes. Cyclosporine was originally used to prevent the rejection of transplanted kidneys, and continues to be used for this as well as for the prevention of graft rejection of a variety of organs. The introduction of cyclosporine (CsA) was a major advance in allograft rejection prevention, reducing acute rejection while increasing patient and graft survival in renal transplant recipients (46). Following oral administration, the absorption and metabolism of cyclosporine are highly variable from patient to patient due to poor gastrointestinal absorption, intestinal transport and first-pass metabolism (47). Cyclosporine is considered to be a narrow therapeutic index drug such that small differences in concentrations can result in lack of efficacy or toxicity. Its clinical use therefore requires frequent therapeutic

drug monitoring. It also is an expensive drug, and the cost–benefit that can be derived from the use of generics is thought to be significant.

Due to the fact that cyclosporine has a narrow therapeutic index and is characterized by variable pharmacokinetics that may change over time (48), some clinicians have argued that BE between two different formulations of this drug should be proven in actual patients rather than in healthy volunteers. Such a study was performed to assess the BE of Pliva's cyclosporine formulation with that of the reference marketed drug, Neoral®. This study has previously been published elsewhere (49). The following will highlight main aspects of the study.

An open-label, multi-center, conversion study completed in 37 stable 6-month post-renal allograft recipients to compare the pharmacokinetics of oral Pliva Cyclosporine Capsules (test) with Neoral (reference). Patients self-administered cyclosporine doses (BID) according to their usual schedule.

The study consisted of three periods. During Period I (Days 1–14), patients were maintained on their usual stable dose of Neoral twice daily. In Period II (Days 15–28), patients switched from Neoral to the equivalent oral dose of Pliva Capsules. Finally, during Period III starting on Day 29, patients returned to their usual dose of oral Neoral. Whole blood samples for cyclosporine pharmacokinetic profiles were collected on Day 1, Day 14, Day 15, Day 28 and Day 29 at the following time intervals: Pre-dose, 0.5, 1.0, 2, 3, 4, 5, 6, 8, 10 and 12 hours post-dose.

Noncompartmental Pharmacokinetic Analysis: Noncompartmental PK analyses were performed using data obtained at steady state only (Days 14 and 28 for the reference and test products, respectively). The AUC during the dosing interval from time 0 to 12 hours at steady-state [$AUC_{0-12}(ss)$] were calculated by the linear trapezoidal method. The maximum blood cyclosporine concentration at steady state [$C_{max(ss)}$] and its corresponding time [$t_{max(ss)}$] were directly obtained from the blood cyclosporine concentration *versus* time profiles. BE determination using noncompartmental PK parameters was based on the parameters $AUC_{0-12}(ss)$ and $C_{max(ss)}$. Point estimates as well as their 90% CIs had to lie between 80% and 125% for BE to be declared.

Compartmental Pharmacokinetic Analysis: This study was performed in patients and only 11 whole blood concentrations could be collected at steady-state in each treatment period per subject. Although this is a large number for a clinical study, this number, as we have seen at the beginning of this chapter, falls short of what is considered to be a rich sampling design for noncompartmental analysis (e.g., typically 15 samples). Compartmental pharmacokinetic analysis therefore had to be performed to ensure the robustness of the results. For that analysis, all whole blood cyclosporine concentrations obtained were utilized. The steady-state data previously used for the noncompartmental analysis and, in addition, two

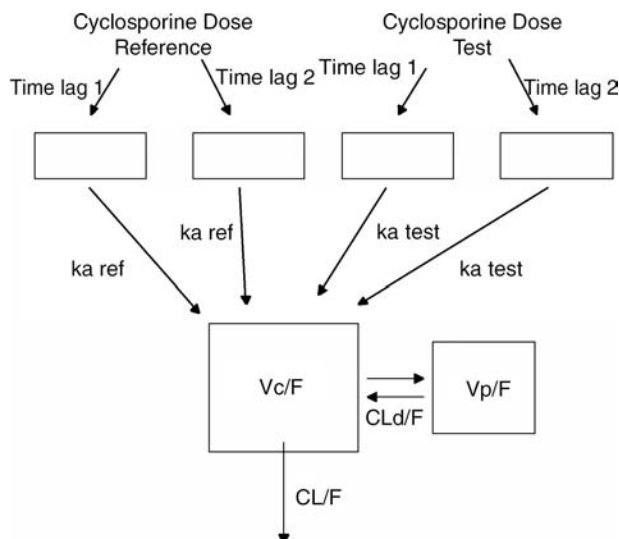


Figure 8 Schematic representation of the two-compartment PK model used for cyclosporine. *Abbreviation:* PK, pharmacokinetic.

other complete profiles obtained the day when patients were switched from one formulation to the other, were used. Cyclosporine concentrations *versus* time were modeled using a mixed-effect modeling population approach (NONMEM[®] version V), and average PK parameters, their inter-individual variability, and the residual variability were calculated. A two-compartment PK model with two absorption peaks arising from a single absorption rate constant and a first-order elimination rate constant was found to be the most appropriate structural model to fit the data, a finding consistent with that which others have also reported for cyclosporine (50–52). This model is presented in Figure 8.

Potential BE differences were assessed by calculating the relative rate constant of absorption (ka_{rel}) and the relative bioavailability (F_{rel}) of the test *versus* the reference cyclosporine formulations.

Results and Discussion

Standard noncompartmental analyses were performed on the concentration *versus* time data that were collected at steady state for the cyclosporine formulations Neoral[®] and Pliva Cyclosporine capsules. Table 8 contains the ratios and their respective CIs for all PK parameters. Based on these results, ratios and 90% CIs were well within 80% to 125%, indicating that the formulations are bioequivalent and switchable in this patient population.

As previously indicated, many clinicians have argued that BE for narrow therapeutic range drugs should be assessed in patients, and have

Table 8 BE Results Using Noncompartmental and Population Compartmental PK Analysis for Cyclosporine in Patients

PK parameters		Ratios (90% CI)	
		Noncompartmental analysis	Population compartmental analysis
Extent of exposure/ Bioavailability	AUC _{0-τ}	98.1 (93–103.6)	N/C
	F _{rel}	N/C	98.4 (96.8–100.0)
Rate of exposure/ Bioavailability	C _{max}	96.9 (88.6–106.1)	N/C
	ka _{rel}	N/C	94.5 (87.6–101.3)

Abbreviations: AUC_{0-τ}, area under the curve during the dosing interval at steady state; C_{max}= maximum observed whole blood concentration during the dosing interval at steady state; CI, confidence interval; F_{rel}, relative bioavailability between the test and reference products; ka_{rel}, relative difference in the absorption rate constant between the test and reference products; N/C, not calculated; PK, pharmacokinetic.

stated that they do not feel comfortable switching formulations of cyclosporine that have been proven to be bioequivalent only in healthy volunteers. This, even when a generic formulation has been approved with implied switchable status by a regulatory agency. The approach presented in this case study clearly validates the findings in healthy volunteers, and shows that the Pliva Cyclosporine Capsule formulation of cyclosporine is bioequivalent to, and as such, switchable with Neoral® in patients. Results obtained in this study using noncompartmental analysis and the population compartmental approach for both rate (e.g., C_{max} and ka_{rel}) and extent (e.g., AUC_{0-t(ss)} and F_{rel}) of exposure measurements were similar in terms of point estimates and CI limits.

Conclusion

The population compartmental approach is very useful when BE is to be assessed in situations where the noncompartmental approach may not be robust, for example when rich sampling is not feasible. Although the latter example with cyclosporine resulted in a similar conclusion using either a noncompartmental or a population compartmental analysis, this may not be the case in a study with sparse or severely sparse data. The population approach presented in the current example should be applicable to other drugs to assess BE in diverse patient populations and under various experimental conditions (e.g., single or multiple doses).

SUMMARY

The noncompartmental PK analysis is the preferred approach to assess BE between two formulations in healthy volunteers for orally administered

drugs and using systemically available concentrations. When the prerequisites for using a noncompartmental PK analysis are not met, however, it becomes necessary to consider other approaches such as population PK modeling to determine BE. It has been shown that such an approach has been successfully applied to assess BE based on urinary output data, when only efficacy data were available for an agent administered by a route other than orally, and when data were available only in patients and not in healthy volunteers.

In a BE setting, the proper use of population modeling in certain instances could be useful to provide information on the comparative rate and extent of exposure/bioavailability of two formulations which may not be available when noncompartmental methods are used.

This chapter presented a limited number of examples where population analyses were used for BE assessment. Furthermore, it is possible to consider many other examples and situations where population analysis would be beneficial for the assessment of BE. It is expected, that like new drug applications, where virtually every submission has now somewhat of a population PK component, this method could have a more important role in future submissions of BE studies especially for drugs administered by a nonoral route or for compounds displaying atypical PK/PD characteristics.

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Role of Metabolites in Bioequivalence Assessment

André Jackson

*Food and Drug Administration, Office of Clinical Pharmacology,
Silver Spring, Maryland, U.S.A.*

INTRODUCTION

Before bioequivalence (BE) became a main part of the biopharmaceutical lexicon, the concept of bioavailability (BA) was established and has been defined in the Code of Federal Regulations (CFR) Sec 320.1 Subchapter D—Drugs for Human Use as follows, “*Bioavailability* is measured by assessing the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action (1)”. For drug products that are not intended to be absorbed into the bloodstream, BA may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action. This emphasis on active moiety suggests that an active metabolite as well as the parent drug may need to be considered in the determination of BA.

Passage of the Drug Price Competition and Patent Term Restoration Act of 1984 (2) created a need to compare the bioavailability of generic and originator (brand name) drug products. Hence, the term, BE was defined in the CFR “the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.”

Clinical pharmacology (CP) addresses the application of pharmacological principles to improve the therapeutic outcome in the practice of

medicine, especially as related to drug therapy and focuses on BA, BE and consequently on safety and efficacy. In some cases the desired clinical response following administration of a drug may also be due to its metabolite(s) such as, for example, amoxapine, which is also an active metabolite of loxapine (3). One can easily see that with the advent of CP and the importance of BA/activity/toxicity, the role of metabolites remains somewhat unclear, especially whenever activity is considered. Unfortunately, some of the philosophy related to moiety activity has permeated BE without a thorough understanding of the potential implications in assessing product performance. However, the major difference between BE and CP is that for BE, the equivalence in the systemic circulation of the amount of parent drug absorbed drug is the pivotal measure and not moiety (i.e., parent and/or metabolite) activity. This is clearly pointed out in the Food and Drug Administration (FDA) Guidance for Industry: BA and BE Studies for Orally Administered Drug Products—General Considerations, issued by the FDA in October 2000 and revised in 2003 (4).

Several authors (5,6) have, however, recommended that metabolite(s) be used for BE determination when any of the following circumstances arise:

1. The parent drug is an inactive pro-drug
2. Plasma concentrations of parent drug are too low to be analyzed
3. Parent drug is metabolized rapidly to an active metabolite
4. Both parent drug and metabolite are active but the metabolite concentration in the systemic circulation is higher

As pointed out in a recent review article, the continuing belief by some that activity is important and should be considered for BE is the major reason for most of the controversy relating to metabolite measurement (6). Other rationales for using metabolites in BE assessment in addition to the activity argument is that metabolite concentrations are generally associated with a lower within subject variability and consequently allows a decrease in the number of subjects required to establish BE. However, analysis of both parent drug and metabolite to assess BE is problematic since it would decrease Type I error (consumer risk) and increase Type II error (producer risk) (3).

METABOLITE FORMATION

The pharmacokinetics (PK) of metabolites as related to BA and BE has been discussed by several authors (6). Therefore, this discussion will briefly review the major types of observed metabolite kinetics.

The PK of a metabolite is characterized by its formation and elimination. Formation of a metabolite can occur via chemical hydrolysis, enzyme-mediated biotransformation, acid/base catalyzed degradation or conjugation. The most common sites of biotransformation of the parent to metabolite

occur in the liver, gut, plasma, kidneys and lungs. If the metabolite is formed pre-systemically, as in the gut or lungs, or in depots, such as in muscle or subcutaneous tissue, the PK of the metabolite is not only governed by its rate of formation, but also by its rate of absorption into the systemic circulation.

SCENARIO 1—METABOLITE FORMATION IS RATE-LIMITING

For a drug administered intravenously with a rate of elimination of parent compound (PC) or formation of metabolite equal (i.e., $k_f/k_{mu}=0.1$; k_f = rate constant for metabolite formation rate; k_{mu} = rate constant for metabolite elimination in the urine). Figure 1 represents formation rate limited kinetics for two cases (Case 1a—intravenous administration and Case 1b—extravascular administration) and indicates that metabolite formation rate is 10 times slower than elimination of metabolite. The kinetics of the metabolite are limited by metabolite formation since it is the slowest step. Typical examples are conjugated metabolites (phase II metabolites), specifically, glucuronides, sulfates or glycine conjugates which are generally more polar than the parent drug and result in faster elimination of the metabolite compared to the parent drug. The same principle applies if the formulation was administered extravascularly, see Figure 1, Extravascular Case 1b, with the following rate constant values (i.e., $k_{dissolution} = (9 \text{ hrs}^{-1})$ rate constant parent drug dissolution; $k_{absorption} = (2 \text{ hrs}^{-1})$ rate constant for parent drug absorption from the gut; $k_f = 0.1 \text{ hr}^{-1}$; $k_{mu} = 1 \text{ hr}^{-1}$) with resulting ratios of: $k_{dissolution}/k_{absorption} = 4.5$; $k_{dissolution}/k_f = 90$; $k_f/k_{mu} = 0.1$. Here, in

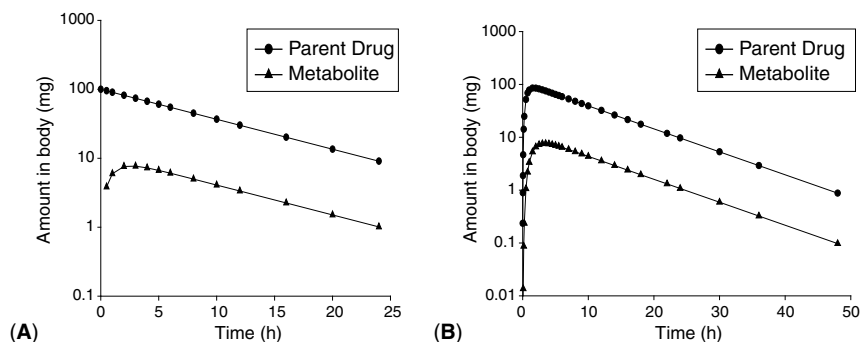


Figure 1 Simulated plasma concentration time profiles of parent drug and metabolite illustrating formation rate-limited kinetics of metabolite following (A) intravenous administration and (B) extravascular administration. *Source:* Adapted from Ref. 6.

addition to metabolite formation being slower than metabolite elimination, dissolution is faster than parent drug absorption, metabolite formation and parent drug elimination.

SCENARIO 2—METABOLITE FORMATION IS RATE LIMITING-IMPACT OF RATE LIMITING DISSOLUTION

Extravascular plots show that whenever absorption becomes the rate determining step graphs can look similar to other extravascular administrations but with some distinct differences. Reference to the plot of PC-time and metabolite-time profiles (Figure 2 which is a composite figure representing Cases 2a and 2b). Case 2a indicates that the terminal slope of the PC profile reflects the rate of dissolution, $k_{\text{dissolution}}$, rather than absorption because it is the slowest process governing PC kinetics. Furthermore, the terminal slope of the metabolite profile reflects $k_{\text{dissolution}}$ not k_f , although k_f is slower than k_{mu} .

On the other hand, Case 2b considers the situation when the slowest step of all processes is the formation of the metabolite, k_f . Although absorption of PC is limited by dissolution rate, the terminal slope of PC *versus* time profile reflects k_f . Similarly, the terminal slope of metabolite profile reflects k_f , although $k_{\text{dissolution}}$ is smaller than $k_{\text{absorption}}$. Note in Figure 2, Case 2b that the C_{max} and AUC of PC have increased 5-fold because of the 5-fold decrease in elimination rate constant, $k_f = 0.2 \text{ hrs}^{-1}$, but the exposure (i.e., area under the curve) of the metabolite remains the same.

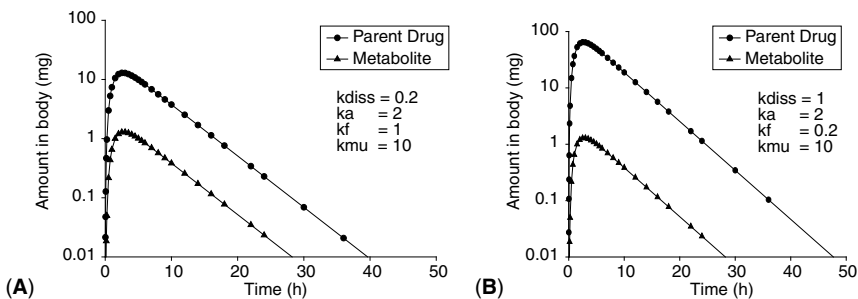


Figure 2 Simulated plasma concentration time profiles of parent drug and metabolite following extravascular administration when metabolite formation is slower than elimination in scenarios (A) dissolution rate limitation and (B) metabolite formation rate limitation. *Source:* Adapted from Ref. 6.

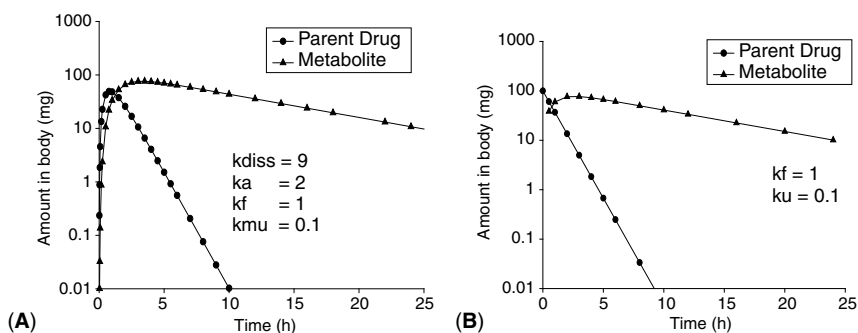


Figure 3 Simulated plasma concentration time profiles of parent drug and metabolite illustrating metabolite elimination rate-limited kinetics following (A) extravascular administration and (B) intravenous administration. *Source:* Adapted from Ref. 6.

SCENARIO 3—METABOLITE ELIMINATION RATE LIMITED KINETICS

In Case 3a, Figure 3 (i.e., extravascular absorption), formation of metabolite is much faster than elimination of metabolite i.e., $k_f \gg k_{mu}$. In contrast in Case 3b, Figure 3 (i.e., intravenous administration), elimination of the metabolite, k_{mu} , limits the kinetics of the metabolite. Metabolism of clozapine to norclozapine (7) is an example of $k_f \gg k_{mu}$ in Phase I metabolism (chemical modification via oxidation/reduction/hydrolysis of PC). An example of $k_f \gg k_{mu}$ in Phase II metabolism (the formation of acetyl conjugates) where the metabolite is generally less polar than the PC and is eliminated slower than the PC (example: procainamide). However, if the dissolution rate is slower than k_{mu} ($k_{dissolution} = 0.5 \text{ hrs}^{-1}$, $k_{mu} = 10 \text{ hrs}^{-1}$), the

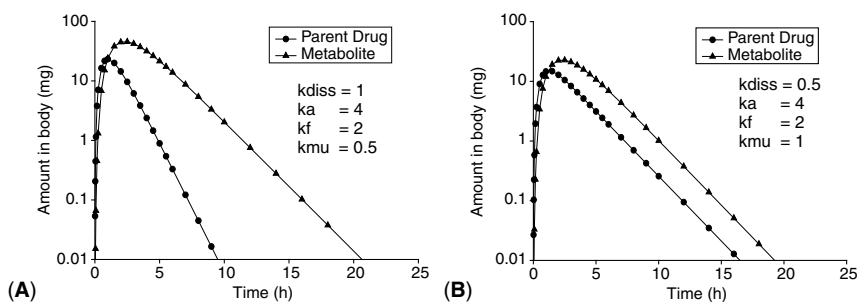


Figure 4 Simulated plasma concentration time profiles of parent drug and metabolite following extravascular administration when metabolite elimination is slower than its formation in scenarios (A) dissolution rate limitation and (B) metabolite elimination slower than dissolution. *Source:* Adapted from Ref. 6.

terminal slopes of both parent and metabolite profiles would reflect k_{diss} (Figure 4, case 4b), the slowest step among all processes involving both PC and metabolite. Figure 4, Case 4a which has k_{mu} as the smallest rate constant reflects the slower elimination of the metabolite.

ROLE OF SIMULATIONS VS. EXPERIMENTAL DATA IN UNDERSTANDING METABOLITES IN BE

A recent paper provided an excellent summary of the advantages of using experimental vs. simulated data to study metabolites in BE (8). The authors point out that with experimental data, the correct answer is always unknown, since there is no way to know if the formulations are truly bioequivalent. On the other hand, with simulation the results are limited only by the validity and assumptions of the models used. The authors summarized the model into two broad categories: (i) simulations seeking to detect the analyte most discriminant for BE based upon the relative widths of the confidence intervals (CI) and (ii) simulations based on intrinsic clearance and the well-stirred model. The authors also did an in-depth comparison of the simulated results with experimental data. Data were first compared with respect to within-subject variability of the hybrid rate constants for parent drug absorption and the hybrid formation rate constant for the metabolite. The second method of comparison evaluated the role of parent drug intrinsic clearance (Cl_{int}) and liver blood flow (Q_H). Simulations showed that when $Cl_{\text{int}} < Q_H$ the parent is preferred for BE analysis and when $Cl_{\text{int}} \geq Q_H$ the metabolite is preferred. Doxepin was cited as an example of the latter. The drug is marketed as an irrational mixture of geometric isomers-15% of the more active *cis* and 85% of the less active *trans* isomer. Due to analytical problems, only 3/30 volunteer subjects in the example had the *cis* isomer analyzable. The authors did some calculations based upon $C_{\text{max}}/\text{AUC}$ ratios and concluded that the greater variability was associated with the parent k_a (rate constant for absorption) and thus the parent should be used. They also pointed out that for this drug $Cl_{\text{int}} > Q_H$ which favors metabolite analysis. These are indeed conflicting conclusions. However, there were two problems with this analysis (i) the parent calculations were based upon total doxepin (i.e., parent + metabolite) which has been previously discussed as being problematic (9) (ii) the small N for the *cis* isomer in the combined analysis. The formulation was not BE with respect to the C_{max} of the parent. Perhaps with a better assay for the *cis* isomer (which would result in a larger N), the CI for C_{max} (within subject variability of 34%) would be decreased, resulting in the parent meeting the CI. The product passed the CI criteria for AUC for both parent and metabolite. For this particular example, the author concluded that based upon reasonable pharmacokinetic and clinical standpoints, the BE decision could be based upon the

active metabolite. However, one must be cautious to be certain that product performance is being evaluated adequately when using the metabolite. Nonetheless, despite the discrepancies between simulated and experimental data in the cited examples, the authors concluded that “any decision to use metabolite data in a given BE study must be made a priori by a drug regulatory agency and should be communicated to the sponsor in the study design stage.” This conclusion clearly favors use of the parent for BE determination unless one has a good reason to use the metabolite.

PRO-DRUGS AND METABOLITES FORMED VIA FIRST PASS

Pro-drugs, by definition, are themselves devoid of intrinsic pharmacological activity, but rather undergo biotransformation to a therapeutically active moiety. The discussion of therapeutic activity again revisits the major problem related to metabolites i.e., are we interested in what is absorbed (making the assumption that if the administered moiety is determined to be BE then activity will be comparable for all species) or does one need to look exclusively at the active species? When analyzing pro-drugs it is useful to distinguish between *post-hoc* and *ad-hoc* design (10). The former is defined as a therapeutically active drug with some unwanted property, which a pro-drug can ameliorate. The therapeutic gain compared to the drug is modest but may have better pharmaceutical properties (i.e., achieve a better therapeutic outcome) if improved targeting is achieved. *Ad hoc* pro-drugs are defined as drugs with some severe drawback in drug properties such as solubility that would restrict BE. This would limit therapeutic use, which again the preparation of a pro-drug overcomes. The *post-hoc* pro-drug clearly complicates the picture related to the choice of active moiety *versus* metabolite since the metabolite is active and has the intrinsic ability to be absorbed, however at a lower BA. This becomes a consideration when one closely examines the possible mechanisms of absorption of the pro-drug.

The current FDA BA/BE guidance states that “The moieties to be measured in biological fluids collected in BA and BE studies are either the active drug ingredient or its active moiety in the administered dosage form (parent drug) and, when appropriate, its active metabolites (21 CFR 320.24 (b) (1) (i)).”

The issue of pro-drugs *versus* metabolites is indeed controversial for two major reasons:

- i. the inactivity of the pro-drug and the common acceptance that the active metabolite is the appropriate analyte for BE and
- ii. reference by the General BA/BE guidance to the formation of a metabolite as a result of gut wall or other pre-systemic metabolism, “[A metabolite may be formed as a result of gut wall or other

pre-systemic metabolism. If the metabolite contributes meaningfully to safety and/or efficacy, we also recommend that the metabolite and the parent drug be measured. When the relative activity of the metabolite is low and does not contribute meaningfully to safety and/or efficacy, it does not have to be measured. We recommend that the parent drug measured in these BE studies be analyzed using a CI approach. The metabolite data can be used to provide supportive evidence of comparable therapeutic outcome.]”

Factor (ii) above is indeed important since the formation of a major, active metabolite during the first pass from the lumen of the gastrointestinal tract to the systemic circulation can be an important issue because formulation factors can influence where the drug dissolves and from which part of the intestine it is absorbed.

The major question related to pro-drugs that could impact metabolites is whether the pro-drug is activated solely in the GI tract, solely in the liver, or in both the GI tract and in the liver? This is a difficult question to address since in most cases it may be a combination of both. A recent review (6) presented a discussion on one of the approaches used for determining the contribution of the gut to the overall first-pass metabolism. It was based upon a study by Paine et al. (11), where the disposition of midazolam and 1-hydroxy-midazolam during surgery in 10 liver transplant recipients in the anhepatic phase was studied. Extraction ratios of midazolam across the gut and liver were found to be 0.43 and 0.47, respectively.

Ideally, if one knew that the pro-drug is indeed absorbed but undergoes first-pass metabolism, then the pro-drug is functioning more like a parent moiety and should be analyzed. If, on the other hand, the parent is metabolized in the gut and the metabolite is absorbed, then the metabolite should be analyzed to determine BE.

Current technology does not allow for any facile method to truly distinguish between gut and liver metabolism, so the currently proposed regulatory procedures related to metabolites do make appropriate provision for the best outcome i.e., looking at the parent drug (whether active or inactive). This gives us an indication of formulation performance, which is a true measure of BE, as well as additional information related to therapeutic effect of the active metabolite, especially if the pro-drug levels are very low or difficult to quantify.

GENOMICS METABOLITES AND BE

The new and developing scientific disciplines of pharmacogenetics, the study of the inherited basis of variable drug response, and pharmacogenomics, the study of inherited differences in inter-individual drug response variability (12), may play a useful role in the study of the role of metabolites in BE.

Metabolic phenotyping involves the administration of a probe drug whose metabolism is solely dependent on the function of a specific enzyme isoform. The metabolites formed are then measured in plasma and urine. This technique could have some use in the design and analysis of BE studies (13). Pharmacogenomic information could be used for:

- i. population enrichment, especially for a parallel-designed study to screen for slow metabolizers
- ii. minimize toxicity from either a toxic metabolite or high dose levels of parent in a population that metabolizes the drug slowly for parallel and crossover BE study designs

The concept of enrichment (14), which is oftentimes used in clinical trials, could also be used in BE studies in cases where the metabolite is of interest for BE assessment (e.g., parent drug not detectable). In most scenarios, the crossover design would account for any intra-subject variation (e.g., a slow metabolizer should have low metabolite levels following ingestion of either the test or the reference formulations). However, there are cases where pre-screening of subjects should be considered. Currently, the design of most BE studies, whether parent and/or metabolite are measured does not usually include pre-screening. This should be considered especially if the drug is contraindicated for a specific genomic subpopulation. A recent report cites the case of codeine metabolism in someone with ultra-rapid CYP2D6 metabolism who developed opioid intoxication (15). Based upon this information, inclusion of this type of patient in a BE study where the metabolite is the compound to be analyzed would not be ethical and hence should be excluded. Surely if one was determining the BE of a compound in which (a) the metabolite is active, (b) the parent is not quantifiable and (c) slow metabolizers give immeasurable levels of metabolite, a major design consideration might be whether to exclude the slow metabolizers, since their plasma levels would be near the limit of quantitation and could add appreciable error to the study results due to their levels being so near the assay limit of quantitation.

A recent article discussed the possibility of using genomic data whenever there is an established relationship between those data and pharmacokinetic data (16). A correlation would have to exist *a priori* between the traditional BE measures $C_{\max}$ and AUC and genetic markers such as DNA sequence, mRNA transcription profiling, linkage and physical maps, gene location, and quantitative trait *loci*. The major problems related to the use of these data would be (a) establishing the correlation and (b) dealing with factors related to model mis-specification. The latter include issues of missing important genomic variables and overall variability. For average BE the difference between the pharmacokinetic and genomic predictions become the basis of any comparison. The authors defined this as:

$$\varepsilon = (\mu_T - \mu_R) - (\hat{\nu}_T - \hat{\nu}_R),$$

where ε —bias is defined by overall difference between pharmacokinetic data prediction (e.g., $C_{\max}$) μ_T —test mean and μ_R —reference mean while $\hat{\nu}_T$ and $\hat{\nu}_R$ are defined respectively as test (T) and reference (R) genetic markers (i.e., DNA sequence, gene location etc.). When $\varepsilon = 0$, residual error, the genomic predictions are unbiased. The tolerance limits for genomic data for average BE are very much dependent upon the amount of variability in the data. The authors also clearly show that differences between the power for average BE PK and genomic data are related to a multiplicative factor defined by the ratio of the standard deviation and the variance measures for the standard two-period two-treatment BE study compared to the same measures in genomic data.

Prior studies have pointed out that the metabolite generally exhibits less intra-subject variability than the parent drug in most BE studies (17). Therefore, based upon the importance of variability in establishing the BE limits and the power of genomic data compared to PK data it is possible to predict the impact of using genomic data for BE. If genomic data are used to compare the difference between parent drug and a metabolite in meeting the BE criteria, establishing BE for the metabolite would require a smaller N , similar to what is currently observed for PK data.

ROLE OF METABOLITE PHARMACODYNAMICS IN DETERMINING BE

An author stated that “The most important role of pharmacodynamics (PD) in BE is not as a substitute for therapeutic equivalence or PK equivalence studies but as a rational basis for defining equivalence criteria” (17). Emphasis on the active species would assuredly decrease the number of parameters to be measured for BE as determined by PD. Due to the large intra-subject variability for PD data, a large N may be required to attain adequate study power. However this would be less of a problem for metabolites because they have been shown to be less variable (18). Nevertheless, one could not be certain that the decrease in PK variability associated with metabolites would also be associated with a decrease in PD variability unless a different PD, (i.e., efficacy response), was observed for parent and metabolite. A larger problem would arise if there were multiple active species, all with the same efficacy endpoint. It has been suggested that using the knowledge of the PD of each active moiety, the predicted time-effect profile of the combination could be predicted. The equivalence of the derived PD parameters used in conjunction with PK of the most active moiety could be used to determine the BE of the product (19). However, the test product would be required to meet several criteria to establish equivalence, thus increasing the possibility of failing the CI requirement

by increasing producer risk. This is known to occur whenever multiple criteria are used to test an hypothesis since each test would be conducted at a lower significance level resulting in a greater chance of products with smaller differences in PK parameters being determined to be not BE (20).

REGULATORY REQUIREMENTS—USE OF METABOLITES IN ASSESSING BE

Health Protection Branch—Canada

The Health Protection Branch Guidelines for Canada in 1992 established the following criteria for immediate-release and modified-release formulations, “The determination of BE is based upon measurement of the active ingredient or its metabolite or both as a function of time. Normally, the PC is sufficient, but in some cases metabolite could be required. When a pro-drug is administered, the active should be measured.”

European Agency for Evaluation of Medicinal Products

The European Agency for Evaluation of Medicinal Products, July 2001, p. 7 states that the applicant must measure the PC. Metabolites are required in the following cases:

1. Concentration of PC is too low, or
2. If PC is unstable or half-life is too short.

The requirements go further to say that if the BE is to be based upon metabolite, it must be justified in each case. In particular, if metabolites significantly contribute to the net activity of an active substance and the PD is non-linear, it is necessary to measure both PC and active metabolite and evaluate them separately.

U.S. Food and Drug Administration

The Guidance for Industry: BA and BE Studies for Orally Administered Drug Products—General Considerations 2003 requests that the PC is measured. Only when a metabolite is formed due to gut wall or other pre-systemic metabolism and the metabolite contributes to safety and efficacy is the metabolite to be measured to provide supportive evidence. In all other instances, only the PC is measured for BE.

As discussed in the introduction to this chapter, the major reason for continued consideration of the role of metabolites in BE and lack of regulatory harmony on this issue 21 years post-Waxman-Hatch Act (i.e., Drug Price Competition and Patent Term Restoration Act of 1984) is the continuing discussion related to the importance of therapeutic activity.

A recent article (8) discussed the impact of allowing the use of multiple analytes for BE determination by different regulatory agencies. Based upon a single parent analyte the consumer risk is set at 5%. If multiple species are requested to be analyzed a decision has to be made *a priori* as to which species will be used for BE determination. The danger with different regulatory agencies having different requirements (i.e., parent *versus* metabolite) is that the consumer risk is reduced but the producer risk is increased since each test is run at a lower alpha level. This needs to be addressed so that uniform BE standards for metabolites can be established worldwide.

Once a clear distinction is made between formulation performance which is measured by BE and activity which BE does not measure and accepted by the scientific community, a more harmonious regulatory worldwide guidance related to metabolites and BE can readily be established.

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Implications of Chirality for the Assessment of Bioequivalence

Reza Mehvar

*School of Pharmacy, Texas Tech University Health Sciences Center,
Amarillo, Texas, U.S.A.*

Fakhreddin Jamali

Faculty of Pharmacy, University of Alberta, Edmonton, Alberta, Canada

INTRODUCTION

Bioequivalence (BE) studies usually consist of a comparison of blood, plasma, or serum drug concentration-time profiles of a test and a reference formulation with regard to the extent and rate of the entry of the active ingredient into the blood circulation. Under certain circumstances, urinary excretion data may also be used (1). Generally, two formulations of the same drug are considered bioequivalent if their measures of extent [area under the plasma concentration-time curve (AUC)] and rate [peak plasma concentration ($C_{\max}$)] of drug entry into the systemic circulation do not differ significantly. The literature over the past several years is replete with theories and guidelines regarding experimental design, type of assay, and data analysis for BE studies of nonracemic pharmaceuticals (2–7). For racemic drugs, often the enantiomers possess different pharmacodynamic or pharmacokinetic characteristics (8–10). Since the 1990s, guidelines have been developed dealing with the BE of racemic drugs as well (11–14), although the issue has still not been quite settled. There are arguments, both in favor and against the need for chiral assays in these studies (15–22). Despite the availability of robust and facile analytical methods for separation and analysis of individual enantiomers, the approach is considered to be laborious and costly compared with the nonstereospecific

analytical procedures. Therefore, justification for requiring chiral assays for BE studies of chiral drugs may best be treated on a case-by-case basis. It is important to note, however, that sometimes applications for the approval of new racemic drugs may be based on the pharmacokinetic, pharmacodynamic, and/or toxicokinetic data for the individual isomers. This, very likely, calls for stereochemical data for approval of generic products regardless of the agencies' guidelines. The currently available guidelines and their limitations with regard to the BE of racemic drugs will be addressed in this chapter.

TERMINOLOGY AND DEFINITIONS

Chirality refers to "handedness" implying the existence of left and right orientations and is the structural characteristic of certain molecules that make them nonsuperimposable on their mirror images (Fig. 1). Molecules with such characteristics are called *chiral* or *asymmetric*.

Enantiomers are one of the examples of chiral structures. *Enantiomers* are either of a pair of substances that are mirror images of each other and that rotate the plane of polarized light equally, but in opposite directions. The most convenient approach in identifying enantiomers is based on dissolving the stereochemically pure enantiomer in a specific solvent followed by testing of the rotation of polarized light that is passed through the solution. The enantiomer is called dextrorotary (d or +) if the light is directed to the right and levorotary (l or -) if the light is rotated to the left. This method does not provide any information regarding the absolute configuration of the enantiomer and the dextro- or levorotary nature will depend upon the conditions used, such as the solvent. Absolute configuration, the three-dimensional structure of the molecule, is denoted by S for sinister (left) and R for rectus (right). An enantiomer with specific absolute configuration may be dextro or levorotary depending upon the experimental conditions that the light rotation is observed. The physicochemical properties of the S and R enantiomers of the same molecule with only one chiral center are identical, however, they often exhibit different biological activities.

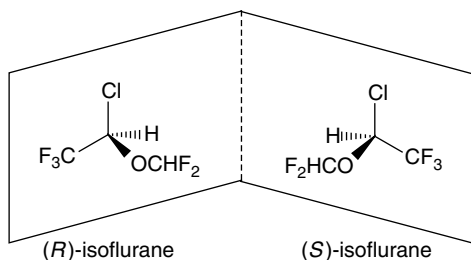


Figure 1 The two nonsuperimposable mirror images of the general anesthetic isoflurane.

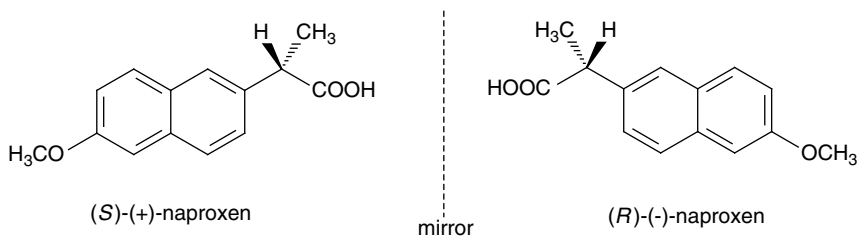


Figure 2 S and R naproxen.

Racemates refer to an equal proportion of a pair of enantiomers, which are optically inactive (Fig. 2).

Racemates usually have different physicochemical properties from the individual enantiomers. Properties of the racemate relative to its enantiomers vary and are based on whether it has formed a *mixture* or a *compound* (23). Often the term “mixture” is erroneously used to identify all racemates, even though racemic compounds are far more common than racemic mixtures (23).

A *diastereoisomer* or *diastereomer* (Fig. 3) is either of a pair of molecules with more than one chiral center that differ with respect to the configurations of their molecules (i.e., stereoisomers) and lack a mirror-image relationship (i.e., are not enantiomers). The individual diastereoisomers differ from each other from the physicochemical viewpoint. This principle is used in analytical chemistry to resolve single center enantiomers from each other for the purpose of quantitation of the individual enantiomers. Indeed, the enantiomers (R and S) of a given racemic drug can be separated if a stereochemically pure reagent (either the R or S enantiomer) is attached to it. This will form diastereoisomers (e.g., RR' and SR' if the R' reagent is used), which will have different physicochemical properties, hence, different retention times on a nonchiral chromatography column.

Racemization occurs when a given enantiomer is turned onto its antipode (the other enantiomer) to form a racemate (equal portions of the two enantiomers). This can happen chemically, e.g., thalidomide (24), or enzymatically, e.g., ketoprofen in the mouse (25). Conversion of one enantiomer to another without resulting in a racemate (i.e., complete or

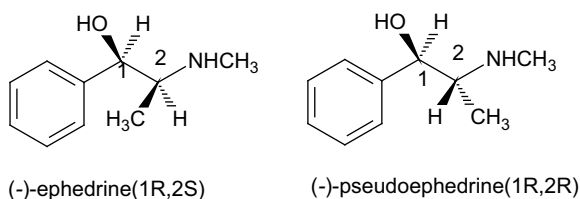


Figure 3 (-)-Ephedrine and (-)-pseudoephedrine are diastereomers.

partial conversion) is conventionally called chiral *inversion*. This usually involves enzymes (e.g., ibuprofen (26)).

Another common terminology error occurs when the term *racemic* is used to describe the concentration of drugs determined using a non-stereospecific assay. This is erroneous since the term *racemate* denotes equal proportions of the enantiomers and the measured concentration may contain different proportions.

The adjective *stereospecific* refers to any process that is specific for one of the two enantiomers, whereas the adjective *stereoselective* refers to a process that favors one enantiomer over the other. For example, if oral absorption of a racemic drug is stereospecific, it means only one of the two enantiomers is absorbed. However, stereoselective absorption means that the absorption process favors one enantiomer over the other. Most pharmacokinetic and pharmacodynamic processes are stereoselective and not stereospecific. Additionally, analytical methods that result in quantitation of the individual enantiomers are stereospecific because they can specifically measure individual enantiomers. However, application of these stereospecific assays will result in stereospecific data that can be stereoselective (i.e., different concentration-time course for the enantiomers). Often, the terms *stereoselective* and *stereospecific* are interchangeably used in error.

REGULATORY GUIDELINES

Most regulatory agencies have either published or follow certain guidelines when it comes to BE studies of racemic drugs. In the following sections, the guidelines for the United States, Canada, European Union, and Japan will be briefly discussed.

United States

The U.S. Food and Drug Administration (FDA) has published (11) a rather straight forward guideline suggesting that a stereochemical approach is NOT required if one of the following conditions applies to the racemic drug:

1. Equal efficacy and/or toxicity is ascribed to the enantiomers, i.e., nonstereoselective effects;
2. Pharmacokinetics of the enantiomers do not differ from each other, i.e., nonstereoselective plasma drug concentration time-course;
3. The “major” pharmacodynamics and/or toxicity effect is ascribed to the predominant enantiomer, e.g., S:R concentration ratio greater than unity and S possesses greater pharmacological activity; or
4. If the enantiomer with lower plasma concentration possesses greater activity but its pharmacokinetics are linear.

Recently (27), Sahajwalla summarized these guidelines in the form of a simple decision tree (Fig. 4). As demonstrated in this figure, the FDA's

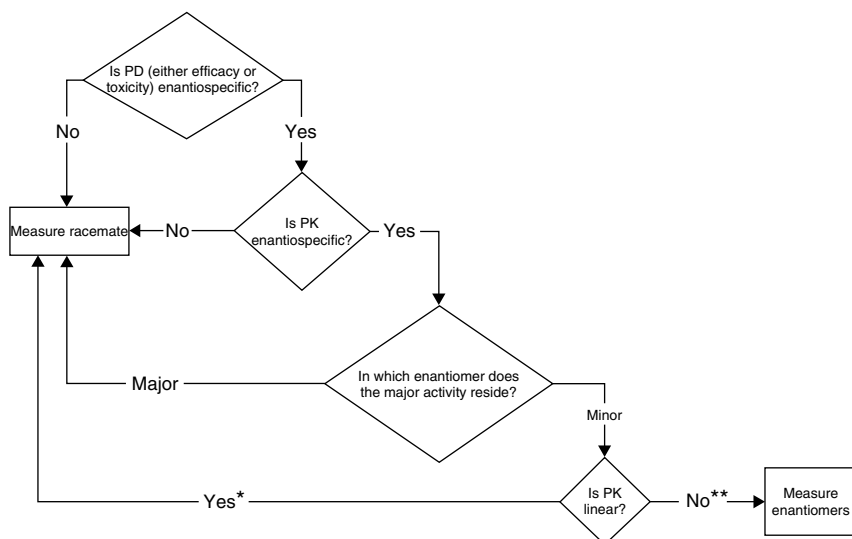


Figure 4 A decision tree for the use of enantiospecific assays based on FDA guidelines. *, Enantiomer ratios remain constant with change in input rate; **, enantiomer ratios change with change in input rate. *Source:* From Ref. 27.

approach is rather cut and dry, but, in general, implies that only on rare occasions will stereospecific data be required. In addition, the guidelines can be challenged based on some observations reported in the literature and will be discussed later.

Canada

The Canadian Guideline published by Health Canada's Therapeutic Product Programme (12) is less detailed than that of the FDA. It states that "In general, when comparing solid oral dosage forms of similar type, e.g., two immediate-release formulations, that contain the same isomeric ratio of medicinal ingredient(s), the parameters to be determined and the standards to be met... will be based on the measurement of total drug concentrations." This implies that for regular release formulations there is no need to present stereospecific data.

The Canadian guideline suggests that for modified release formulations, however, there may be occasions where enantiospecific data may be required. These include situations when "comparative bioavailability" studies are conducted to compare solid oral dosage forms of "differing type", e.g., a modified-release *versus* an immediate-release product, or *versus* a "different kind of modified release formulation." Stereospecific analysis may also be required when "the rate of release and/or absorption of the

medicinal ingredient into the systemic circulation affects the in vivo enantiomeric ratio (e.g., drugs with enantioselective nonlinear first pass metabolism).”

The Canadian guideline cannot be considered helpful from the pragmatic viewpoint because of the following reasons:

1. BE studies hardly are conducted to test a regular *versus* modified release formulations.
2. The information regarding effect of the absorption rate on the enantiomeric ratio is hardly available when application for a generic product is filed. It implies that the lack of information regarding the issue suggests a lack of dependence of the enantiomeric ratio on the absorption rate. Alternatively, it may imply that the onus is on the sponsor of the generic product to confirm lack of dependence of enantiomeric ratio on the absorption rate.

Europe

The European guideline (13) states that BE studies supporting generic product applications of chiral medicinal products should be based upon enantiospecific bioanalytical methods, unless (a) both products contain the same, stable single enantiomer as the active substance or (b) both products contain the racemate and both enantiomers show linear pharmacokinetics.

Japan

The Japanese regulatory agency does not suggest stereospecific assay unless when there “exist stereoisomers with different activities for the main pharmacological effect,” and when stereoselective absorption or elimination, or absorption rate-dependent stereoselectivity is noticed (14). In these cases the enantiomer with higher activity should be measured.

From the above discussion it becomes evident that no two regulatory agencies are in general agreement as how to assess the BE of racemic drugs. In such cases, taking the most stringent approach is, perhaps, most advisable.

LIMITATIONS OF THE GUIDELINES

A careful examination of the above guidelines indicates that they are all based on the categorization of drug products with reference to the degree of stereoselectivity in their pharmacodynamics and pharmacokinetics and/or the presence or absence of linear pharmacokinetics. However, it is hard to find a racemic drug for which the properties of both enantiomers are thoroughly reported. As discussed in the following sections, these issues are

not always clear, hence, decisions based on the criteria stated in various guidelines may be challenged.

Stereoselectivity in Pharmacodynamics and Pharmacokinetics

As mentioned above, both FDA and Japanese guidelines state that when the enantiomers of a racemic drug exhibit equal efficacy and/or toxicity, BE studies based on the measurement of the total drug will be sufficient. However, in some cases, despite equipotency of the main pharmacologic activity, the enantiomers may differ in other, desired or undesired, effects (Table 1) (8,28). For instance, various studies (28–30) have shown that the two enantiomers of propafenone (Table 1) elicit a similar degree of antiarrhythmic effect. However, the *S*-enantiomer is more potent (>40 fold) than its antipode as a β -blocking agent (28,29,31). To complicate the matter further, it has been suggested that propafenone enantiomers may possess differences in their antiarrhythmic effects as well (30). Nonetheless, if these other effects of the so-called equipotent enantiomers of chiral drugs are important components of the overall therapeutic or toxic activities of the racemate, then measurement of the individual enantiomers may be necessary in certain cases.

Lack of stereoselectivity in pharmacokinetics is another reason for exemption of a requirement for stereospecific data for some regulatory

Table 1 Relative Pharmacologic Activities of the Enantiomers of Some Racemic Drugs

Relative activity	Experimental model	Biological response
Bucindolol		
– = +	Rat	α -Blockade, vasodilation
– > + (250:1)	Rat	β -Blockade
Disopyramide		
+ = –	Humans	Negative inotropic, diastolic effects
+ > –	Humans	Prolongation of QTc duration
Meptazinol		
– = +	Guinea pig ileum	Opioid agonist activity
– > +	Guinea pig ileum	Cholinergic properties
Propafenone		
S = R	Humans	Antiarrhythmic
S > R	Human lymphocyte	β -blocking activity
Secobarbital		
S = R	Mouse	Anticonvulsant
S > R	Mouse	Anesthetic

Source: Data from Ref. 8 with the exception of propafenone data in human lymphocyte which were taken from Ref. 28.

agencies. Indeed, literature data show that the plasma concentration-time courses of the enantiomers of some drugs are not substantially different from one another under certain experimental conditions (Table 2) (32–42). Obviously, BE studies of these drugs do not require the use of stereospecific assays. A study on the BE of flurbiprofen (43) is in agreement with this guideline. Although there was a small difference in S-flurbiprofen between the two products, the confidence intervals for the two products were within 80% to 125% in terms of both individual enantiomers and total drug.

However, a lack of stereoselectivity in the pharmacokinetics in one population does not guarantee similar profiles under different circumstances (e.g., disease status, age, drug interactions, patient populations). This situation is more frequently observed when pharmacogenetics plays a role in the disposition of the racemic drug. For instance, whereas the (+):(-) AUC ratio of pantoprazole was close to unity (0.82) in extensive metabolizers (CYP2C19) of mephenytoin, the ratio was 3.59 in poor metabolizers (44). A less drastic pharmacogenetics effect has been reported for flecainide, where the average R:S ratio of trough concentrations in the plasma of patients with arrhythmia was 1.1 (Table 2) (36). However, another study (45) indicated

Table 2 Enantiomeric AUC Ratios of Some Racemic Drugs Exhibiting Minimal Stereoselectivity in AUCs after Oral Administration

Drug	AUC ratio	Population studied	Reference
Acebutolol	1.2 (S:R)	Young healthy volunteers	(32)
	1.2 (S:R)	Elderly volunteers	(33)
Atenolol	1.1 (R:S)	Healthy volunteers	(34)
Ethosuximide	1.1 ^a	Epileptic patients	(35)
Flecainide	1.1 (R:S) ^b	Patients with arrhythmia	(36)
Flurbiprofen	1.1 (S:R)	Healthy volunteers	(37)
Ketoprofen	1.2 (R:S)	Healthy volunteers, SD	(38)
	1.1 (R:S)	Healthy volunteers, MD	(38)
	1.0 (R:S)	Young and elderly arthritics, SD and MD	(38)
Mexiletine	1.0 and 1.1 (S:R)	EMs and PMs of debrisoquine	(39)
Sotalol	1.0 (R:S)	Healthy volunteers, SD	(40)
	1.1 (+:-)	Patients with arrhythmia, MD	(41)
Tiaprofenic acid	1.0 (S:R)	Arthritic patients	(42)

^a First: second chromatographic peak concentration in plasma at various times after the administration of different doses of the drug.

^b Steady-state trough concentration ratio.

Abbreviations: EMs, extensive metabolizers; MD, multiple dose; PMs, poor metabolizers; SD, single dose.

that the stereoselectivity in plasma concentrations of flecainide is dependent on the debrisoquine/sparteine metabolizer phenotype; while the extensive metabolizers (EMs) had an R:S AUC ratio of 1.0, the ratio was 1.3 for poor metabolizers (PMs). Therefore, relatively extensive studies in different patient populations may be needed before a drug can be safely labeled as not demonstrating stereoselectivity in its kinetics. The lack of stereoselectivity in the AUCs of ketoprofen enantiomers under various circumstances (Table 2) (38) suggests that one can safely label this drug as such. Indeed, 90% confidence interval analysis of data (20) from a BE study (46) of two enteric-coated formulations of ketoprofen is consistent with this conclusion.

The degree of stereoselectivity in the pharmacokinetic parameters of two enantiomers of a racemate may also be affected by disease states. Upon administration of racemic ibuprofen to healthy subjects, the serum concentration of the pharmacologically active S enantiomer is predominant mainly due to bioinversion of R to S. It has been shown (47), however, that acute pain results in reversal of the serum ibuprofen enantiomers concentration ratio (Fig. 5). This may be a result of a change in the metabolic chiral inversion of R-ibuprofen to the pharmacologically more important S enantiomer. This process is therapeutically significant as it constitutes 60% of the total clearance of R-ibuprofen (48) and thus may augment pharmacological activity through formation of active S-ibuprofen. Reversed stereoselectivity in plasma ibuprofen concentrations has also been observed in two other groups of patients, namely adults with liver cirrhosis (49) and infants (6–18 months) recovering from minor genito-urinary surgery (50). Both of these patient groups are likely to have reduced biotransformation capacities compared to healthy adults. It is not known whether the reversal of the S:R plasma concentration ratio in infants is age-related or, as it has been observed in adults, is caused by surgery. Delayed absorption of ibuprofen was also observed in these infants (50).

Nevertheless, it may be argued that altered stereoselectivity in specific conditions is likely due to the post-absorption processes, hence, once BE is demonstrated in one patient population, it can be extrapolated to others. However, in light of the more recent understanding regarding the involvement of gastrointestinal transporters and enzymes, the issue is not quite settled. For example, for some arylpropionic acid derivatives of nonsteroidal anti-inflammatory drugs, such as ibuprofen (51) and fenoprofen (52), the R to S bioinversion, takes place in both the gut and liver. The inversion in the gut is negligible for immediate release formulations. However, it becomes significant following administration of slow-release formulations designed to release their active ingredient in the gut (53–55). This renders such drugs as good candidates for inclusion among those that require a stereochemical approach in assessment of their BE. Indeed, a report (56) on the effects of food on the BE of ibuprofen shows how complexities in the inversion and/or absorption of enantiomers may affect the outcome. In this study, the fasted

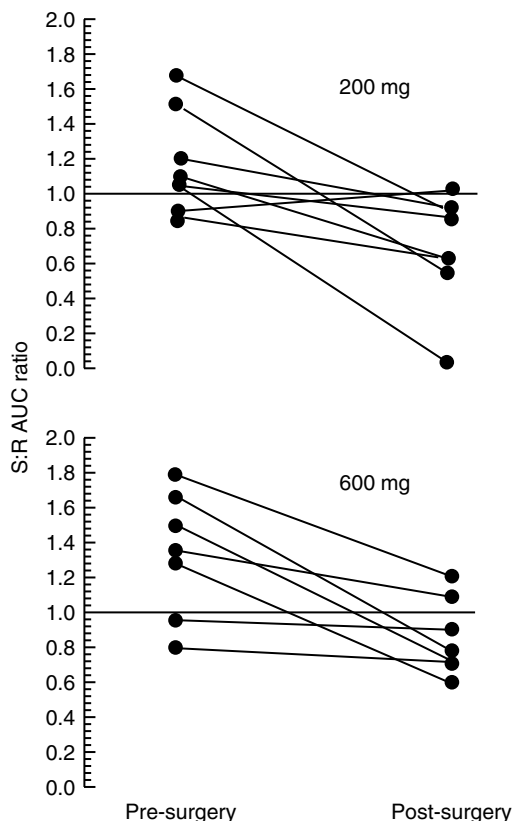


Figure 5 The S:R AUC ratios of ibuprofen in individual patients after administration of either 200 mg or 600 mg of racemic ibuprofen one week before surgery (pre-surgery) and at the time of first experience of moderate to severe pain (post-surgery). *Abbreviation:* AUC, Area under the plasma concentration-time curve. *Source:* From Ref. 47.

and fed situations were not bioequivalent in their $C_{\max}$ (lower for fed). However, when the individual enantiomers were considered, the drug was bioequivalent in the two situations with regard to the active S-enantiomer; the food only affected the BE of R-ibuprofen.

The antihistaminic effect of racemic chlorpheniramine is ascribed mainly to the S enantiomer. S-chlorpheniramine is also the predominant enantiomer in plasma. A BE study (57) confirmed BE between two products based on nonstereoselective data. However, based on stereospecific approach, S but not R enantiomer achieved BE. According to the four guidelines discussed in this chapter, stereospecific data are not required for the assessment of chlorpheniramine products. In addition, since both the total

(S+R) and the pharmacologically active S-enantiomer confirmed BE, one is inclined to suggest that the observed lack of BE for the R-chlorpheniramine should not be taken into account. Such a notion is based on the assumption that the R enantiomer is, indeed, devoid of activity and/or there is no interaction between the enantiomers, hence, its bioinequivalence is unlikely to have any pharmacological or toxicity consequences. This, however, may not always hold true as the presence of the 'inactive' enantiomer may result in altered therapeutic consequences as shown for some of the nonsteroidal anti-inflammatory drugs with respect to gastrointestinal side effect (58).

Linearity in Pharmacokinetics

Linear pharmacokinetics is an exemption criterion for stereospecific data requirement for most regulatory agencies, such as the FDA, Canadian and European authorities. Theoretically, the stereoselectivity in the AUC of racemic drugs with linear kinetics should be independent of the input rate and formulation of the drug. Therefore, the enantiomeric AUC ratios should remain constant for different formulations and doses. This implies that the extent of availability of the active enantiomer is directly related to the extent of the availability of the total drug. However, the differences between two formulations with regard to $C_{\max}$ and the time to reach $C_{\max}$ ($T_{\max}$) could be dependent on the measured entity (total drug vs. individual enantiomers). This is particularly important when differences in pharmacokinetic parameters of two enantiomers are large. Previously-reported (19) simulations for drugs with large enantiomeric differences in their kinetics (such as acenocoumarol (59), etodolac (60), hexobarbital (61), and mephobarbital (62)) well illustrated this concept; substantial stereoselectivity (e.g., 5-fold) in either clearance or volume of distribution resulted in different BE decisions for $C_{\max}$ based on the stereospecificity of the assay used. However, the AUC values were not affected by the rate of input.

Available data in the literature also support our suggestion that BE conclusions for drugs with linear pharmacokinetics and large stereoselectivity in pharmacokinetics may be dependent on the specificity of the assay. Consider the following examples of drugs with linear pharmacokinetics, one with modest and the other with substantial stereoselectivity in their pharmacokinetics. Hydroxychloroquine is a drug with linear pharmacokinetics and relatively small degree of stereoselectivity in its pharmacokinetics (R:S blood AUC ratio of 1.8) (63,64). Tett et al. (64) showed that the concentrations of the two enantiomers of the drug were highly correlated ($r^2 = 0.889$) across a wide concentration range resulting from different doses and interindividual variability in pharmacokinetics among 43 patients with rheumatoid arthritis. In agreement with these observations, a subsequent parallel-design BE study by Midha et al. (65) demonstrated BE of two formulations of the drug based on both the total drug and the individual

enantiomers. These data support the exemption guidelines for a drug with linear pharmacokinetics, but, as previously stated, only when stereoselectivity in pharmacokinetics is relatively small.

The second example is etodolac (60), which we previously (19) identified as a racemic drug with linear, but substantially stereoselective, pharmacokinetics for which BE studies based on the total drug may be misleading. Indeed, Boni et al. (66) confirmed our suggestion with regard to the BE of etodolac in a later publication. When two treatments of etodolac solutions mimicking two formulations with different rates of absorption were administered, different BE conclusions were made using total *versus* S-etodolac data. In their study, Treatments 1 and 2 both consisted of a single 400 mg dose of racemic etodolac solution administered at once (Treatment 1) or as five 80-mg doses administered every eight minutes (Treatment 2) in a crossover study, reducing the rate of absorption in the latter. The BE test results for the $C_{\max}$ and AUC of the two treatments (Table 3) were consistent with our prediction. Whereas the two “formulations” were bioequivalent with respect to the $C_{\max}$ of total etodolac, lack of BE was concluded based on the $C_{\max}$ of the more clinically relevant S-etodolac (Table 3). Additionally, similar to our simulations (19), the BE conclusion for the AUC was the same regardless of the measured entity (Table 3).

Therefore, the BE of racemic drugs with substantial pharmacokinetic differences between enantiomers may result in different conclusions based on stereospecific or nonstereospecific analytical methods even in the presence of linear pharmacokinetics.

Nonlinearity in Pharmacokinetics

Based on the FDA guidelines (11), nonlinearity in pharmacokinetics is a requirement for stereospecific data only if the enantiomer with lower plasma concentration possesses greater activity. In contrast to the FDA guidelines (11), the European (13) and Canadian (12) guidelines are more clear in that

Table 3 Bioequivalence Data for $C_{\max}$ and AUC for Two Rates of Release of Etodolac

Parameter	$C_{\max}$		AUC	
	(R, S)-Etodolac	(S)-Etodolac	(R, S)-Etodolac	(S)-Etodolac
Geometric mean ratio	102	120	102	102
90% CI	96–109	108–134	96–108	96–107
Bioequivalence	Yes	No	Yes	Yes

Abbreviations: AUC, Area under the plasma concentration-time curve; $C_{\max}$, peak plasma concentration.

Source: Data from Ref. 66.

nonlinear pharmacokinetics are generally regarded as grounds for stereospecific data requirement. Although nonlinearity in pharmacokinetics may arise at the levels of absorption, distribution, and/or elimination, it normally is a result of saturable or Michaelis-Menten (MM) behavior. For racemic drugs exhibiting nonlinear kinetics, the enantiomeric ratio of the blood concentrations or AUCs could be dependent on the oral input rate of the drug to the systemic circulation (67–72). Therefore, BE of two products based on total drug concentrations may not necessarily infer BE of the individual enantiomers.

Nonlinear Oral Absorption

If the enantiomers of a racemic drug are absorbed through the gastrointestinal tract by a saturable stereoselective mechanism, BE studies based on total drug may not be directly extrapolated to the individual enantiomers. As an example, a set of simulated data for a racemic drug with stereoselective and nonlinear absorption has been reported (19), which indicates that substantial differences in the $C_{\max}$ and $T_{\max}$ of two formulations for one enantiomer may not be reflected in the kinetic parameters of total drug. Therefore, a decision on the BE of the two products based on total drug may not be valid for a drug with nonlinear absorption. Based on these data, it is clear that the BE of chiral drugs exhibiting stereoselective absorption should be carried out using stereospecific analytical methods. Stereoselective absorption has been suggested for chiral β -lactam antibiotics (73) and stiripentol (74).

Nonlinear Clearance

Kinetically, enzymatic metabolism of all drugs can be described by MM behavior. However, for most drugs, the therapeutic doses are substantially lower than the maximum rate of metabolism ($V_{\max}$). Therefore, their metabolism can follow linear kinetics. On the other hand, for some drugs, the input rate may approach the $V_{\max}$ of the drug. This becomes especially important during oral absorption, when large amounts of the drug enter the liver through the portal vein. For these drugs, the enantiomeric plasma concentration or AUC ratios may be affected by the input rate (formulation release rate) of the drug into the systemic circulation (67,68). The results of a simulation study (19) using estimated MM parameters and volume of distribution of verapamil (VER) enantiomers showed that a formulation-dependent drastic change in the plasma concentration-time profile of the active *S*-enantiomer was attenuated when the total drug was measured. This was because the *R*-enantiomer, which is predominant in the plasma, was less susceptible to the changes in the rate of absorption. Consequently, it was suggested that the BE decisions for chiral drug with nonlinear, stereoselective metabolism could be dependent on whether stereospecific or non-stereospecific assays are used.

Experimental evidence also exists for the absorption rate dependent changes in the degree of stereoselectivity of VER pharmacokinetics. For examples, a study (70) in humans using a single 240-mg oral dose of racemic VER, administered as immediate-release and sustained-release formulations revealed R:S plasma concentration ratios which were dependent on the formulation; the ratios at $C_{\max}$, and several other time points, were lower for the immediate release product (4.52), compared with the sustained-release formulation (5.83). Additionally, R:S AUC ratios for the sustained-release formulation (7.75) were larger than those for the immediate-release formulation (5.04) (70). The evidence, however, for such a formulation-dependent stereoselectivity in the $C_{\max}$ and AUC values of VER for two formulations with less drastic differences in the rate of input remains scarce. In a book chapter, Longstreth (75) reported that based on an achiral assay, two sustained-release formulations of VER were considered bioequivalent. However, analysis of the individual enantiomers in a subset (12 patients) of the study population (24 patients) resulted in the rejection of BE.

Similarly, in another report (71) in humans it was shown that the S:R ratio of $C_{\max}$ for a single 160 mg oral dose of immediate release propranolol (1.44) was slightly, but significantly, lower than that observed after 80 mg immediate release or 160 mg controlled release formulations (1.54). However, the S:R AUC ratios were not input rate dependent. For drugs exhibiting MM metabolism, input rate-dependent stereoselective pharmacokinetics are expected to be substantial when nonlinear kinetics are operative, i.e., when the daily dosing rate approaches the $V_{\max}$ of the enantiomers (67,68). Therefore, input rate-dependent stereoselective pharmacokinetics are more likely at high blood concentrations of the enantiomers. The lack of input rate dependency of the AUC ratios for propranolol in the above study (71) may have been due to the fact that in this study, single doses of relatively low magnitude were administered, resulting in blood concentrations closer to the linear range.

Based on the above arguments, it appears prudent to use a stereospecific assay in BE studies of chiral drugs that show nonlinear elimination pathways at therapeutic doses. This is especially important if the so-called therapeutic doses are close to the maximum rate of metabolism of the enantiomers. Examples of such drugs are VER and propranolol.

The enantiomeric plasma concentration ratios of racemic drugs with other nonlinear, stereoselective elimination pathways (such as renal elimination) can be similarly influenced by the oral input rate (or formulation) of the drug.

Nonlinear Plasma Protein and/or Tissue Binding

Similar to other nonlinear, stereoselective kinetic processes mentioned above, nonlinearity in the protein or tissue binding of enantiomers can potentially make the drug susceptible to input rate-dependent alterations in

the stereoselectivity of plasma concentrations of drug. Especially, for drugs undergoing significant first-pass metabolism, nonlinear protein binding in the portal blood during the absorption phase may result in alterations in the blood concentration ratios of the enantiomers. For these drugs, therefore, BE studies should be based on the individual enantiomers.

OTHER FACTORS INFLUENCING BE OF CHIRAL DRUGS

In addition to the above pharmacokinetic and pharmacodynamic considerations, the BE of chiral drugs may be influenced by other factors listed below.

Formulation Issues

Excipients

Many excipients used in the formulation of drugs are optically active. Therefore, theoretically, these chiral excipients may interact with the optically active drug in the formulation and result in stereoselective release of the chiral drug. Indeed, some reports (76–81) have shown that such stereoselective releases are experimentally possible. However, in all of these cases, stereoselectivity in the release has been insignificant (82). Therefore, based on the available data, it appears that the impact of excipients on the stereoselective kinetics of chiral drugs, if any, is minimal.

The greatest extent of stereoselective release has been observed in vitro with a commercially available sustained release capsule of tiaprofenic acid (Surgam SR, Roussel, Montreal, Canada) which exhibited up to 29% stereoselectivity at pH 7.4 (79). The observed in vitro stereoselectivity, however, did not result in differences in any bioavailability indices of tiaprofenic acid enantiomers following administration to healthy human volunteers. The discrepancy between the in vitro and in vivo results has been attributed to the failure of the dissolution test to predict bioavailability outcomes. Nevertheless, the possibility of stereoselective interactions with excipients should be considered during the development stage.

Modified Release Formulations

Compared with immediate release formulations, modified release products are inherently more susceptible to input rate dependent alterations in their stereoselective pharmacokinetics. This is because the in vivo release of most modified release formulations is a complex process often comprising of a combination of zero and first order kinetics whose pattern may not be directly predicted from the available in vitro release tests. Therefore, in the presence of input rate dependent stereoselective pharmacokinetics (e.g., nonlinear kinetics and/or high degree of stereoselective pharmacokinetics), an erroneous BE decision based on total drug is more likely for modified release formulations.

STATISTICAL AND ANALYTICAL ISSUES

In addition to revealing stereoselective processes, stereospecific analytical methods inherently possess greater statistical power than do methods based on total drug. This is due to the fact that the differences in only one enantiomer become smaller when calculated as a percentage of total drug than when expressed in terms of a single enantiomer concentration. For example, an observed 38% difference in the $C_{\max}$ of S-ibuprofen constitutes only a 27% difference when expressed as a *percentage* of total drug (83). This is true only if the precision, accuracy, and sensitivity of the two methods are comparable. Therefore, the comparison of BE results based on stereospecific and nonstereospecific methods may be complicated by differences in the characteristics of the analytical methods. A possible example is a study (84) of the BE of nadolol, a β -blocker marketed as two racemate pairs each consisting of two enantiomers (a total of four stereoisomers) with differences in potency of the stereoisomers. In that study, the BE of two formulations of nadolol were investigated using both stereospecific and nonstereospecific assays and single-dose and steady-state data. Using both steady-state and single-dose data, the two formulations were bioequivalent for total drug. However, BE was rejected based on all four individual enantiomers at steady state and also based on three enantiomers using the single dose data. Additionally, the authors showed some nonlinearity in the kinetics of the drug because AUCs at steady state were higher than those predicted from the single dose data. A limitation of this study was that the sample size was based on the variability in the kinetics of the total drug, which was found to be less than those for the individual enantiomers. However, a later analysis of data (20) suggested that the apparently higher variability in the pharmacokinetics of individual enantiomers in comparison with that for total drug might have been due to a possibly higher variability of the stereospecific assay, compared with the nonstereospecific method. Because of the statistical and analytical questions raised in this study, one cannot be certain whether the different BE conclusions based on the total and individual enantiomers are indeed due to the relatively minor nonlinearity in the kinetics of the drug or from an artifact of assay differences.

CONCLUSIONS AND FUTURE DIRECTIONS

The currently available guidelines and their limitations with regard to the BE of racemic drugs have been reviewed and discussed in this chapter. Although theoretical and experimental data support some components of the guidelines, others are not supported by the available experimental and/or theoretical data. Nevertheless, clear and reliable experimental data supporting or refuting the current guidelines are scarce because most of these studies have not been designed to test these guidelines. Therefore, variables

such as differences in the characteristics of stereospecific and non-stereospecific assays and also variability in the pharmacokinetics of individual enantiomers and total drug have not been controlled in these studies. Future studies should employ chiral and achiral assays that have similar precision and accuracy characteristics. Additionally, the lower limit of quantitation of each assay should be commensurate with the lowest level of each measured moiety (enantiomers *vs.* total drug for the lowest expected plasma concentrations). Furthermore, it should be noted that studies which have used two formulations with very close rates and extent of availability intuitively should result in the same conclusion based on either individual enantiomers or total drug. Therefore, future studies should be designed with formulations that have differences close to the boundaries of 80% and 125% to test the differences between the chiral *versus* nonchiral assay methods.

It is worthy of re-emphasizing that applications for the approval of new racemic drugs may contain stereochemical information. Hence, it may call for stereochemical data for approval of generic products regardless of the various guidelines. In such cases, it is unclear whether BE will be based on one or all optical isomers. Based on the forgoing discussion, however, it is wise to take all isomers into account.

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Effect of Food on Bioavailability and the Assessment of Bioequivalence

Kim Dalhoff

Clinical Pharmacologic Unit, Bispebjerg Hospital, Copenhagen, Denmark

Isadore Kanfer

*Faculty of Pharmacy, Rhodes University,
Grahamstown, South Africa*

BACKGROUND

It is important to recognize and prevent food–drug interactions since failure to do so may be hazardous to patients and predispose them to treatment failure, toxicity or even life-threatening adverse events. The increasing complexity of drug therapy regimens has increased the risk of food–drug interactions, and it is important that healthcare professionals are aware of the mechanisms behind the interactions and also realize the advantages and disadvantages of methods to prevent food–drug interactions in hospitalized patients. Introduction of computerized prescription of drugs in more and more hospitals may lead to systems that are capable of screening and warning about serious interactions before the drug is administered to the patient. Standard drug administration schedules, hospital newsletters, educational in-services, label systems and patient counselling are other measures which can help avoiding food–drug interactions (1). Furthermore, evaluating the effect of food on the absorption and bioavailability of drugs is an essential requirement in drug development.

MECHANISMS OF FOOD–DRUG INTERACTIONS

Physical and chemical characteristics of a drug are important factors in considering the potential for interaction with food. Different drugs within the same drug group or different formulations of identical drugs can have different physicochemical characteristics thereby resulting in completely

different food–drug interactions. The food effect is least likely to occur with rapidly dissolving, immediate-release drugs containing highly soluble and highly permeable drug substances because absorption of such drugs is usually pH and site independent and hence insensitive to differences in dissolution.

Food–drug interactions may also be related to characteristics of the meal. Size and composition of the meal as well as the exact timing of drug intake in relation to the meal are important determinants of interactions (2). The bioavailability of lipophilic drugs is often increased by a high fat content, either because of increased drug solubility or stimulation of bile secretion. Alternatively, high fibre content may reduce the bioavailability of certain drugs because of binding to the fibres. In this context there is no exact definition of fasting, but it normally means no food intake for at least 1 hour before and at least 2 hours after drug intake.

The small intestine is the major drug absorption site and many physiological factors may influence drug absorption. For example, amongst these factors are:

1. stomach and intestinal pH
2. gastrointestinal motility
3. presence of material on the luminal site of the bowel (e.g., food acting as a physical barrier between the drug and the mucosal surface)
4. the number of absorbing cells lining the gastrointestinal tract and the rate of splanchnic blood flow.

Changes in gastric pH caused by food or age may affect tablet disintegration. Aspirin and isoniazide are examples of drugs whose rate of disintegration is affected by pH resulting in delayed or reduced absorption (3). Gastric emptying may also be affected by the presence of food. In the fasting state drugs generally leave the stomach quickly and a delay in gastric emptying may, in fact, promote the absorption of some drugs where delays could permit such drugs to be dissolved before passing on to the small intestine for subsequent absorption, for example, erythromycin tablets or capsules (4). However, in contrast, other drugs, such as amoxicillin and ampicillin, for example (5) may be destroyed by the prolonged retention time in the stomach. Increased splanchnic blood flow, resulting from food intake, may also influence the absorption of drugs that are extensively metabolized as a result of changes in the clearance of the drug during first-pass passage through the hepatoportal system.

PHARMACOKINETIC AND CLINICAL EFFECTS

Since the effect of drugs is influenced by bioavailability, a change in bioavailability is an important consideration in food–drug interactions. The most important pharmacokinetic food–drug interactions are caused by changes in the absorption of a drug because of chemical reactions between

the drug and food (e.g., chelation) or to the physiological response to food intake, which mainly cause changes in gastric pH, bile secretion or gastrointestinal motility. Food–drug interactions that only affect the rate of drug absorption are common but rarely of clinical importance. However, with some drugs, rapid absorption resulting in high peak drug concentrations may be undesirable because of the development of concentration-dependent adverse effects. The relevance of a food induced change in bioavailability can only be evaluated if the impact of the food on the pharmacological effect is quantified by studies which include relevant clinical effect parameters, such as measurement of blood pressure by antihypertensive drugs, for example. However, for many drugs the pharmacological effect is not directly quantifiable. Several reviews give guiding recommendations for administration of drugs with significant and clinically relevant food–drug interactions e.g., Refs. 3 and 6.

DRUG-DIETARY SUPPLEMENTS AND DRUG-HERB INTERACTIONS

The increasing use of dietary supplements including herbal remedies presents a special challenge in the management of a patient's health care. St. John's wort (*hypericum perforatum*), a dietary supplement often used for depression (7) is one of the top-selling dietary supplements in the United States and the purported active substance, hyperforin, is a potent inducer of CYP3A enzymes (8). Following reports of serious interactions between St. John's wort and indinavir and St. John's wort and cyclosporine, the U.S. Food and Drug Administration (FDA) issued a public health advisory in 2000 with a warning for the concomitant use of such drugs with St. John's wort. The combination resulted in reduced concentrations of indinavir and cyclosporine respectively, and serious complications leading to therapeutic failure. Since 2000 an increasing number of reports have been published implicating the possible role of St. John's wort in the varied response of several therapeutic agents including oral contraceptives (breakthrough bleeding and pregnancy).

Another top-selling product, Echinacea, often used for the treatment of cold/viral infection, may affect the activity of drug metabolizing enzymes. Studies in healthy human subjects show a substantial change in CYP activity after administration of 400 mg Echinacea 4 times a day for 8 days. This effect is most pronounced for CYP3A activity suggesting that special precautions may be taken if Echinacea is taken together with a drug that is a substrate for CYP3A.

Ginkgo biloba is often used for memory improvements, and this product may also cause serious drug-herb interactions. In vitro studies show that Ginkgo biloba affects the activity of metabolizing enzymes e.g., induction of CYP2C19. Consequently drugs, which are substrates for CYP2C19, may lose their therapeutic effect if taken together with Ginkgo biloba.

DRUG–CITRUS FRUIT INTERACTIONS

Grapefruit juice and drug interactions have been investigated extensively for many years and are the best examples of drug–nutrient interactions exclusively caused by inhibition of intestinal CYP3A4 (9). Oral absorption pharmacokinetic studies of CYP3A4 substrates (cyclosporine or felodipine) consistently showed that grapefruit juice increased oral bioavailability of these drugs. A study from 1997 (10) showed that repeated consumption of grapefruit juice inhibits not only the intestinal CYP3A4, but also the expression of the gene in the enterocytes. Dose reduction of the affected drugs, especially those with narrow therapeutic indices and serious side effects, may be necessary to avoid toxicity.

Other fruit juices, apple, calcium-fortified orange juice and cranberry juice have been studied less extensively than grapefruit juice. In particular, calcium-fortified orange juice may change the pharmacokinetics of fluoroquinolones, but it is still unclear whether it is associated with a significant clinical effect, i.e., therapeutic failure. However, the current labelling for ciprofloxacin tablets has information for patients with a warning that concomitant use of the drug with calcium-fortified orange juice should be avoided since the absorption of ciprofloxacin may be significantly reduced.

INFLUENCE OF FOOD ON DRUG ABSORPTION, DISPOSITION AND PHARMACOKINETICS

Bioavailability studies and food effects are usually conducted during the development of new drugs intended for oral administration in order to assess how the rate and extent of absorption of the drug are affected when the drug is administered shortly after a meal compared with administration under fasting conditions. Also fed bioequivalence (BE) studies may be necessary to assess generic drugs in order to demonstrate their BE to a reference comparator drug.

Food may affect drug absorption/bioavailability in several different ways. The bioavailability of many antimicrobial agents is reduced when administered with food. Cephalosporins, penicillin G and penicillin V are examples of antibiotics where the area under the blood-level *versus* time curve (AUC) are significantly decreased in the fed state compared with the fasting state (11). Also, peak aspirin levels are reduced in the region of 40% to 50% by food together with a significant reduction in the rate of absorption (12). Food may also delay the absorption of drugs such as with some anti-inflammatory drugs. Alclofenac absorption was delayed in healthy subjects when administered immediately before and 30 min after a standard breakfast (13).

The clinical significance of any such food effect is however largely dependent on the type of drug therapy and the magnitude of an associated

change. The width of a drug's therapeutic window, potency, pharmacokinetic and pharmacodynamic properties (dose-response relationship) and potential for serious toxicity/adverse events, largely dictate the need to take necessary precautions in the labelling and use of such medications likely to be associated with clinically significant outcomes. Importantly, when undertaking a comparative bioavailability study (BE assessment) of a generic product, it is necessary to establish that any clinically significant food effect associated with the reference (innovator) product is the same as that of the test product (generic). However, several questions regarding the need to assess the effect of food need to be answered before embarking on a BE study. For example, what criteria are used to indicate that a fed study is required? Should a fed study be carried out in addition to a fasting study or will a fed study alone suffice?

Within the last few years' regulatory authorities throughout the world have released guidelines, which more or less explicitly recommend how and when to conduct food-effect bioavailability and BE studies. In 2002 the U.S. FDA released a very thorough guidance (14) and Health Canada followed in 2005 by releasing a document concerning the subject (15), together with a notice which defined which drugs should be subject to food effect studies. A new category of drugs, critical dose drugs, has recently replaced the category of narrow therapeutic range drugs and highly toxic drugs (16). In Europe, the European Agency for the Evaluation of Medicinal Products (EMA) has a more general guideline on the investigation of bioavailability and BE (17). However, this guideline only briefly comments on the methodology of bioavailability and BE studies for assessing food-drug interactions. In Australia, the Therapeutic Goods Administration (TGA) adopted the EU guideline in 2002 (18).

According to the FDA guidance (14), both fasted and fed studies should be conducted for all new chemical entities. The FDA also recommends that a BE study under both fasting and fed conditions be conducted for all orally administered immediate-release drug products, with the following exceptions:

- When both test and reference listed drug (RLD) products are rapidly dissolving, have similar dissolution profiles, and contain a drug substance with high solubility and high permeability (BCS Class I) (19,20),
- When the dosage and administration section of the RLD label states that the product should be taken only on an empty stomach, or
- When the RLD label does not make any statements about the effect of food on absorption or administration.

FDA, further recommends that both fed and fasting BE studies are carried out for all modified-release dosage forms.

Health Canada's published guidance for industry (15) makes the following recommendations:

- For uncomplicated immediate-release oral drug products, BE study under fasting conditions
- For complicated drugs in immediate-release oral drug products (narrow therapeutic range and highly toxic – now known as Critical Dose Drugs (16) and non-linear drugs) BE study under both fasted and fed conditions required
- For ALL controlled/modified release dosage forms—Fast and Fed BE study required

The EMEA (17) requires that if the summary of product characteristics of the comparator product contain specific recommendations in relation to food intake or related to food interaction effects, the study should be designed accordingly.

In Australia, the TGA recommends both fasted and fed studies for any drug product known to exhibit differences in absorption and disposition due to food (18).

A more realistic and practical approach has been proposed by the South African regulatory authority, the Medicines Control Council. As with other international regulatory authorities, only fasting studies are required for immediate-release oral dosage forms and both fasting and fed studies are required for all controlled/modified release dosage forms. However, if a drug product is labeled to be taken with food, only a fed study need be conducted (21,22).

Toothaker and Welling (23) described various drug – food interactions according to classes of interactions. They provided tables showing drug products whose absorption may be reduced, delayed or increased by food. The specific dosage forms of each product together with type of food and time interval relative to dosing were included. Also tabulated were a list of drug products that were apparently unaffected by food.

Winstanley and Orme (24) provided a list of drugs whose bioavailabilities can be altered to a clinically important degree by food. The table included drugs whose bioavailabilities were increased or decreased by food.

The following table (Table 1) provides “food-effect” data for some drug products whose patents have or are soon to expire, making them potential candidates for generic drug product development.

Wu and Benet (194) expanded on the findings of Fleisher et al. (195) who noted that food effects on the extent of bioavailability could be generally predicted on BCS class. Based on the premise that food effects result from changes in drug solubility and other factors (19) they hypothesized that drug-transporter interactions could often be the primary mechanism for a food effect. They contended that high fat meals may inhibit both influx and

(Text continues on p. 217.)

Table 1 Food Effects on the Pharmacokinetics of Various Drugs

Drug name	Bioavailability (<i>F</i>)	Absorption	$C_{\max}$	$T_{\max}$	AUC	References
6-Mercaptopurine	Decrease		Lower	Increase	Decrease	25–27
Acetaminophen	No effect	No effect	No effect	No effect	No effect	28,29
Acyclovir		Decrease				30
Alendronate	Decrease	Decrease				31
Almotriptan	No effect	No effect	No effect	No effect	No effect	32–34
Amitriptyline	No effect					35
Amlodipine	No effect					36,37
Atenolol	Decrease			Decrease	Decrease	38
Atorvastatin	No effect	No effect	Lower	Increase		37,39–42
Azithromycin	No effect		Higher		Higher	43–47
Bisoprolol	No effect					48,49
Bromazepam			Lower	Increase	Lower	50
Bromocriptine	No effect					51
Budesonide	No effect					52,53
Bumetanide	No effect		Lower	Increase		54
Bupropion	No effect					55,56
Buspirone			Increase		Increase	57
Cabergoline	No effect		No effect	No effect	No effect	58–60
Carbamazepine	No effect				Increase	61–63
Carvedilol	No effect					64
Cefaclor	No effect	Decrease	Decrease	Increase		65–67
Cefixime	No effect					66,68–70

(Continued)

Table 1 Food Effects on the Pharmacokinetics of Various Drugs (*Continued*)

Drug name	Bioavailability (<i>F</i>)	Absorption	$C_{\max}$	$T_{\max}$	AUC	References
Cefpodoxime	Increase	Increase	Increase	No effect	Increase	71–76
Cefprozil	No effect		No effect	Increase	No effect	65,77
Cefuroxime	Increase		increase		Increase	78–81
Celecoxib	No effect					82
Cephalexin	No effect					83,84
Cilazapril	No effect		Decrease	Increase	Decrease	85
Cimetidine	No effect					86,87
Ciprofloxacin	No effect					88
Clarithromycin	No effect					89
Clavulanic acid	No effect					90
Clopidogrel	No effect					91
Ethinyl estradiol	No effect					92
Didanosine	Decrease		Decrease	No effect	Decrease	6,93–96
Diltiazem	No effect	No effect	No effect	No effect	No effect	97,99
Doxycycline	No effect	Decrease				23,100–102
Enalapril	No effect	No effect				103
Eprosartan	No effect	Decrease	Decrease			104
Famciclovir	No effect					105
Famotidine	Decrease					106,107
Felodipine	No effect					108,109
Fenofibrate	Increase/no effect		Increase		Increase	110,111
Fexofenadine						112
Finasteride	No effect					113

Fluconazole	No effect		No effect		No effect	114–118
Fluvoxamine	No effect		No effect	No effect	Decrease	119
Gabapentin	Increase/no effect	Increase				120,121
Ganciclovir	Increase	Increase	Increase	Increase	Increase	122–124
Gatifloxacin	No effect	No effect	No effect	No effect	No effect	125,126
Glimepiride	No effect					127
Glipizide	No effect	No effect	No effect		No effect	128
Glyburide	No effect					129,130
Granisetron	No effect					131,132
Itraconazole	Increase	Increase	Increase		Increase	6,115–117,133–137
Ketoprofen	Decrease/no effect	Decrease	Dcrease		Decrease	138–141
Lamotrigine	Decrease	Decrease	Decrease		Decrease	142
Lansoprazole	Decrease	Decrease	Decrease	Increase	Decrease	143
Levetiracetam	No effect	Decrease	Decrease	Longer	Decrease	144
Levonorgestrel/Ethinyl Estradiol	No effect					92
Lisinopril	No effect					145,146
Losartan	No effect					147
Lovastatin	Increases					6
Meloxicam	No effect					148
Metformin	No effect	No effect	No effect	No effect	No effect	129,149
Mirtazapine	No effect	No effect	No effect	Increase	No effect	150
Misoprostol acid/diclofenac	Decrease	Decrease	Decrease	Increase	No effect	151
Moexipril						152
Nefazodone	No effect	Decrease	Decrease	No effect	Decrease	153,154
Nifedipine	No effect	Decrease	Decrease	Increase	Decrease	155–157
Omeprazole	No effect	Decrease	No effect		No effect	158,159

(Continued)

Table 1 Food Effects on the Pharmacokinetics of Various Drugs (*Continued*)

Drug name	Bioavailability (<i>F</i>)	Absorption	$C_{\max}$	$T_{\max}$	AUC	References
Oxaprozin	No effect	No effect	No effect	No effect	No effect	160
Pantoprazole	No effect					161
Pentoxifylline	No effect					162
Pioglitazone	No effect					163
Pravastatin	No effect					164
Prazosin	No effect					165
Quinapril	No effect					166,167
Rabeprazole	No effect					168,169
Rosiglitazone	No effect	Slight decrease	Decrease	No effect	No effect	170
Roxithromycin	No effect					171
Sirolimus	No effect					172–174
Tacrolimus	Increase					6,175
Tamsulosin	No effect					176,177
Terazosin	Decrease	Decrease	Decrease	Increase	No effect	178,179
Terbinafine						180
Ticlopidine	Increase			Decrease	Increase	181,182
Tolterodine	No effect					183,184
Topiramate	No effect	Decrease				185–188
Tramadol	No effect					189,190
Trazodone			Decrease	Increase	No effect	191
Venlafaxine	No effect	Decrease				192
Ziprasidone	Increase					193

efflux drug transporters and that their preliminary studies suggest that a high fat meal will inhibit P-glycoprotein. However, high fat meals will have no significant effect on the extent of bioavailability for BCS class I compounds because complete absorption may be expected for high solubility/high permeability drugs since no transporter drug interactions would be expected for such compounds. It was however emphasized that high fat meals may delay stomach emptying and thus cause an increase in $T_{\max}$.

High fat meals are however likely to increase the extent of bioavailability due to inhibition of efflux transporters in the intestine and additional solubilization of drug in the intestinal lumen. Hence $T_{\max}$ could decrease due to efflux cycling or increase due to slowing of stomach emptying and suggested that a combination of the two mechanisms will usually be dominated by the delayed emptying. The issue is further complicated by the possibility that high fat meals could inhibit both uptake and efflux transporters, and depending on the relative magnitude of inhibition of uptake and efflux transporters, food effects may be confounding and more likely to have little effect on the extent of bioavailability, yet still resulting in an increase in $T_{\max}$ due to delayed gastric emptying.

If formulation changes markedly increase the solubility of Class II compounds, such changes will decrease or eliminate the effect of high fat meals for this class of drugs. This is purported to be the reason that the formulation of cyclosporine as a microemulsion (Neoral) has eliminated the food effects associated with the earlier olive oil formulation (Sandimmune).

Finally, they suggested that high fat meals will decrease the extent of absorption for BCS Class III drugs due to inhibition of uptake transporters in the intestine. However, they noted that some Class III drugs can be substrates for intestinal efflux transporters and depending upon whether the food effects are more pronounced on efflux or influx transporters for a Class II drug that is a substrate for both, an unexpected increase in the extent of bioavailability or no food effect may be observed as is the case of a lack of a high fat meal effect on acyclovir. Generally, for Class II drugs, $T_{\max}$ would be expected to increase with a high fat meal due to the combination of delayed stomach emptying and consequently slower absorption.

Whilst it is difficult to predict/explain the effect of food on Class IV drugs, it was suggested that if a high fat meal interaction were to occur, an increase in the extent of bioavailability is more likely.

Utilization of different formulation principles including controlled release tablets or capsules often improves patient compliance since the frequency of drug intake is reduced. They may also produce a relatively constant plasma concentration, beneficial in the control of the underlying disease (e.g., control of blood pressure). However, sometimes food impairs the ability of a formulation to release the drug in a regulated manner with serious toxicity consequences as a result of dose dumping from rapid and uncontrolled increase in drug concentration in the systemic circulation,

which could be due to gastric retention. This may delay the release of the drug since absorption in the stomach is slow. When the dissolved active substance is finally emptied into the small intestine, it becomes absorbed rapidly and any controlled release mechanism previously built into the formulation will have been negated. This may consequently give rise to multiple peaks in the plasma concentration-time profile. Dose dumping was believed to cause the significant difference in nifedipine pharmacokinetics in 24 healthy subjects treated with nifedipine Sandoz Retard under fed and fasting conditions (196).

THE U.S. FDA

Food-effect bioavailability information should be available and conducted early in the drug development process. The FDA recommends that food-effect bioavailability studies are conducted for all new chemical entities—both immediate-release and modified-release drug products. For already approved drug products that require *in vivo* re-documentation of BE under fasting conditions, it is generally not necessary to conduct food–drug interaction studies in the fed state. The FDA recommends that a food–drug interaction bioavailability study for new drugs should be designed as a randomized, balanced, single-dose, two-treatment (fed *vs.* fasting), two-period, two-sequence crossover study. A similar design should be applied when conducting a fed BE study (test *vs.* reference drug) for generic drug products. Such studies should be carried out in healthy volunteers, but if safety concerns preclude enrolment of healthy subjects, a patient population can be used. A minimum of 12 subjects should complete the food–drug interaction bioavailability and fed BE studies. The highest strength of the drug intended to be marketed should be tested in such studies. However, lower strengths of the dosage form can be used if clinical safety concerns prevent the use of the highest strength. The FDA also gives recommendations on how to prepare a test meal for use in food-effect bioavailability and BE studies. Meal conditions should provide the highest effect on gastrointestinal physiology so that systemic drug availability is affected maximally. It is recommended that the test meal should be rich in calories (approximately 800–1000 calories) and fat (approximately 50% of total calorie content of the meal). Protein and carbohydrate content of the test meal should derive approximately 150 and 250 calories, respectively and the calorie breakdown should be provided in a report following the study.

Administration of the standardized meals in relation to drug intake is also described in the FDA guideline. Following an overnight fast of at least 10 hours, the subjects should start the test meal 30 min prior to administration of the drug product and the meal should be finished within 30 minutes. The drug product should then be given with 240 mL of water. After drug intake no food should be allowed for at least 4 hours post-dose. In each

period of the study the test meal should be administered at the same time of the day.

To characterize the drug concentration-time profile of the drug product, plasma samples should be collected from the study subjects in both fasted and fed treatment periods. Consideration of sample collection times is important in food-drug interaction studies, bearing in mind that co-administration of a drug with food can alter the time course of plasma drug concentrations so that fasted and fed treatments can have different sample collection times. The resulting concentration-time curves should derive pharmacokinetic parameters and in the FDA guideline relevant exposure measures are listed:

- Total exposure or area under the concentration-time curve ($AUC_{0-\text{inf}}$ or AUC_{0-t})
- Peak exposure (C_{max})
- Time to peak exposure (T_{max})
- Lag-time (t_{lag}) for modified-release products, if present
- Terminal elimination half-life
- Other relevant pharmacokinetic parameters

How to analyse and report the exposure measurements are described in detail. Log transformation of AUC and C_{max} prior to analysis is recommended and 90% confidence intervals (CI) for the ratio of population geometric means between test and reference products should be used for $AUC_{0-\text{inf}}$, AUC_{0-t} and C_{max} .

An absence of food-effect on bioavailability is indicated when the 90% CI for the ratio of population geometric means between fed and fasted treatments is contained in the equivalence limits of 80% to 125% for $AUC_{0-\text{inf}}$ (AUC_{0-t} when appropriate) and C_{max} and the label following the drug product should describe this effect accordingly (e.g., the drug product may be taken with no regard to meals).

HEALTH CANADA

Since small differences in the amount of a “critical dose drug”^a available to the body may result in consequences more serious than with “uncomplicated” drugs, a greater degree of assurance of similarity between reference and the generic (test) drug product is required. For “critical dose drugs” differences in mean AUC between test and reference formulation require a 90% CI within an acceptance interval of 90% to 112%. The 90% CI of the

^aDefined as those drugs where comparatively small differences in dose or concentration lead to dose- and concentration-dependent, serious therapeutic failures and/or serious adverse drug reactions which may be persistent, irreversible, slowly reversible, or life threatening events.

relative mean measured $C_{\max}$ of the test to reference formulation should be between 80% and 125%, the same equivalence limits as in the FDA guideline. These requirements are to be met in both the fasted and fed states according to Health Canada.

CONCLUSIONS

Food-drug interactions may have serious consequences for patients either by development of drug toxicity or by inducing treatment failure and it is important for clinicians to be aware of mechanisms and the clinical effects of food-drug interactions. The pharmacokinetic profiles of almost all drugs are different if taken with food compared to the fasting state. However, it is only a relatively small number of drug in which it may have serious clinical implications to ignore warnings of food-drug interactions. Specifically, the effect of food on bioavailability is an extremely important consideration for the registration and market approval of generic drug products and health authorities all around the world have, within the last few years, published guidelines with specific requirements for food-effect bioavailability and BE studies.

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Bioequivalence Assessment of Endogenous Drug Substances: Pharmacokinetics and Statistical Evaluation

Philippe Colucci

*Faculté de Pharmacie, University of Montreal,
Montreal, Quebec, Canada*

Marika Pasternyk-Di Marco

*Pharmacokinetics and Pharmacodynamics, MDS Pharma Services, Montreal,
Quebec, Canada*

Diane Potvin

Theratechnologies, Inc., Montreal, Quebec, Canada

Murray P. Ducharme

*Cetero Research, Cary, North Carolina, U.S.A. and
Faculté de Pharmacie, University of Montreal, Montreal, Quebec, Canada*

INTRODUCTION

This chapter describes the challenges faced when evaluating the bioavailability and bioequivalence (BE) of orally administered compounds that are endogenously present in the body.

The increased importance of biotechnology in the pharmaceutical industry has been attracting renewed attention to endogenous substances. Endogenous substances have been previously defined in a multiple of ways (1–3). However, the simplest definition describing these substances is that they are present and naturally synthesized in the body and are usually

controlled through mechanisms that maintain a state of equilibrium or balance in the body (called homeostasis) (1,3–5). Endogenous substances play important physiological and biochemical roles in the biological organism and when impaired can often be restored through the intake of appropriate foods containing specific exogenous compounds and/or dietary supplements or administration of drugs (5–7).

The relationship between pharmacokinetics (PK, simply defined as “what the body does to a drug”) and pharmacodynamics (PD, simply defined as “what the drug does to the body”) of endogenous substances is complex. Usually, a therapeutic response can be correlated with the amount of drug that is delivered to the site of action or biophase. However, in the case of endogenous substances, because they occur naturally in the body, exogenously administered endogenous compounds will coexist with the body’s endogenous substances and interact at the sites of activity or toxicity.

Generic drug products can be demonstrated to be pharmaceutically and therapeutically equivalent to an innovator product by generally conducting *in vivo* BE studies. These BE studies are difficult to perform for exogenously administered endogenous substances because of substantial variability in the concentrations of endogenous substances in the body (7). There is therefore a baseline concentration of endogenous substances present prior to the administration of the exogenous compound, and this baseline may not be stable because of normal homeostasis principles and also because the PK behavior of some endogenous products may be complicated by inter-conversion with metabolites, special metabolic pathways and/or non-linear attributes (5,7,8).

This chapter focuses on the challenges facing the PK and statistical assessment of endogenous substances administered exogenously. Three specific approaches in determining the endogenous baseline as well as the factors affecting baseline stability will be discussed. Finally, specific case studies related to demonstrating BE between Test and Reference formulations of orally administered endogenous substances will be presented.

DETERMINATION OF THE ENDOGENOUS BASELINE

The majority of BE studies for non-endogenous compounds (“xenobiotics”) are performed in healthy volunteers that are “naïve” to the administration of the test and reference products. In addition, these studies are usually designed to administer both the test and reference compounds in a crossover fashion where provision is made for an adequate washout period (e.g., typically 7 to 10 terminal half lives). In these circumstances, it is easy to determine the exact systemic contribution of the administered compound because the pre-dose concentrations will be undetectable and assumed to be zero. This is represented in [Figure 1](#).

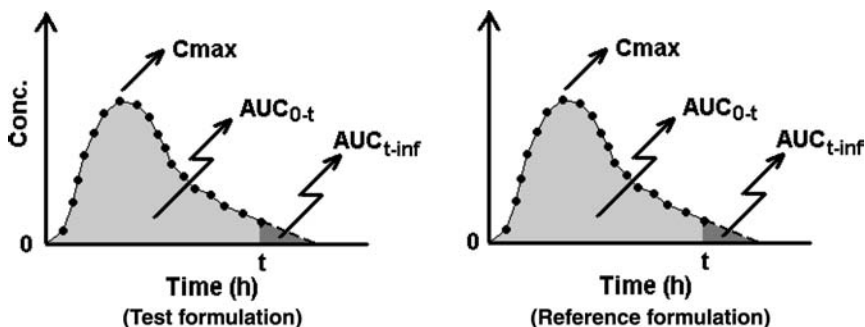


Figure 1 The exposure to an exogenously administered xenobiotic is typically easily captured since there should not be any presence of the administered drug in the systemic circulation prior to administration.

The situation is of course, very different for endogenous compounds administered exogenously. For these, establishing the baseline is a critical step to ensure that the PK behavior of what is exogenously administered will be robustly assessed. In terms of BE, we must exclude the presence of the endogenously produced compounds. It is therefore crucial to separate those substances that are present naturally in the body from those which are exogenously administered.

Three different situations of baseline endogenous concentrations can occur.

- The baseline endogenous concentrations can be stable.
- The baseline can be unstable in a predictable manner (e.g., circadian rhythm in the baseline endogenous concentrations).
- The baseline can be unpredictably unstable or stable because of homeostatic equilibrium operating through feedback mechanisms in the body preventing major fluctuations (increases or decreases) in the systemic concentrations of the endogenous substance.

Stable Baseline

Endogenous baseline concentrations appear to be constant and stable despite the administration of supplemental exogenous compounds. This situation is represented in [Figure 2](#).

The contribution resulting from the administration of the exogenous compound can therefore easily be computed by subtracting the baseline area from the observed (exogenous + endogenous) exposition. However, three difficulties need to be dealt with which can be described as “rules” in the PKs of endogenous compounds. The first rule is that the baseline concentration has to be as robustly characterized as possible. Observed concentrations measured in

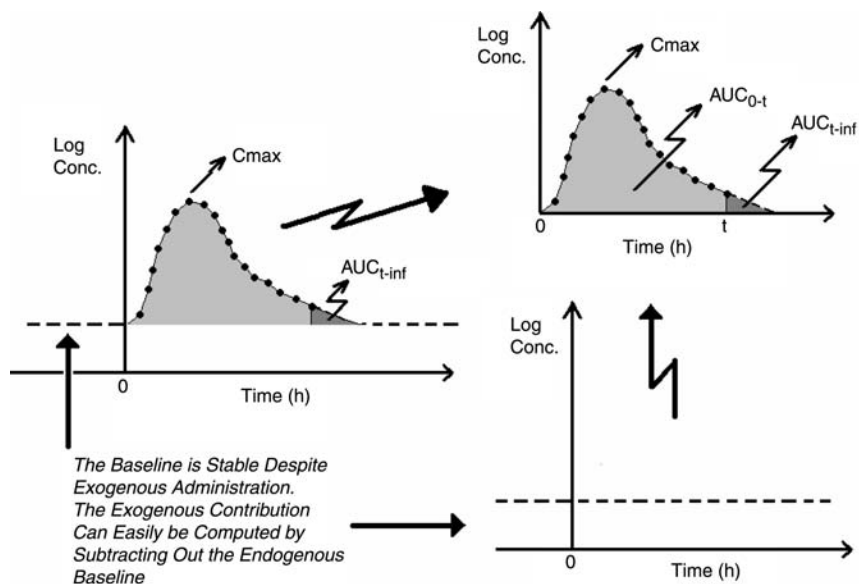


Figure 2 Exposure to an exogenously administered endogenous drug is easily computed when the endogenous baseline is stable.

a BE study are typically associated with 5% to 15% uncertainty. This uncertainty derives from the experimental errors associated with performance of the study (e.g., errors in the administered dose, in the timing of the blood draws, etc.), which are typically small in such a Phase I clinical environment, and are also due to the variability associated with the bioanalytical method itself, usually less than 15%. If we assume that this overall variability is 15%, one can understand that the baseline concentration of endogenous substance cannot be reliably assessed simply from one pre-dose concentration. This would result in a 15% uncertainty in the measurement of the baseline, an unacceptable error in the context of a BE study where two formulations need to be proven to be equivalent in terms of real exogenous exposure and maximum concentrations using the standard BE acceptance criteria. However, by measuring more than 1 pre-dose baseline concentration, this uncertainty can be reduced. As a general rule, a minimum of 3 to 5 pre-dose concentration measurements can ensure that the uncertainty in the baseline assessment is kept to a minimum, at approximately 5%.

The second and third rules deal with the calculation of the adjusted exposure (AUC corrected (AUC_{corr}), also termed AUC adjusted (AUC_{adj}). The mean value of the baseline concentration must be subtracted from every post-dose concentration. Because of the 15% uncertainty previously mentioned in any measured concentration, this can result in some adjusted concentrations becoming negative instead of being either positive or zero

(e.g., in the absence of any uncertainty in the measured concentrations). These negative values have to be retained for the PK analysis and the adjustment has to be subject- and period-specific. If negative values are artificially adjusted to zero, then the corrected or adjusted AUC will be overestimated. The pre-dose or 0-hour concentration also needs to be set to zero (in other words to the mean endogenous concentrations) in the adjusted data set. In order to avoid any errors in the calculation of the corrected AUC, it should be verified and calculated as:

$AUC_{0-t(\text{corrected})} = AUC_{0-t(\text{observed})} - (\text{Baseline} \times t)$. These three rules are presented in Figure 3.

Examples of endogenous concentrations for which the baseline levels are stable are those before and following the administration of several sexual hormonal drugs (e.g., estrogen, progesterone, etc.). A case study related to this is discussed at the end of this chapter.

Unstable But Predictable Baseline

These situations occur for endogenous compounds that are influenced by circadian rhythm. Testosterone in young healthy males and steroids compounds are typical examples. An example of circadian fluctuation is seen for cortisol where higher concentrations occur in the morning followed

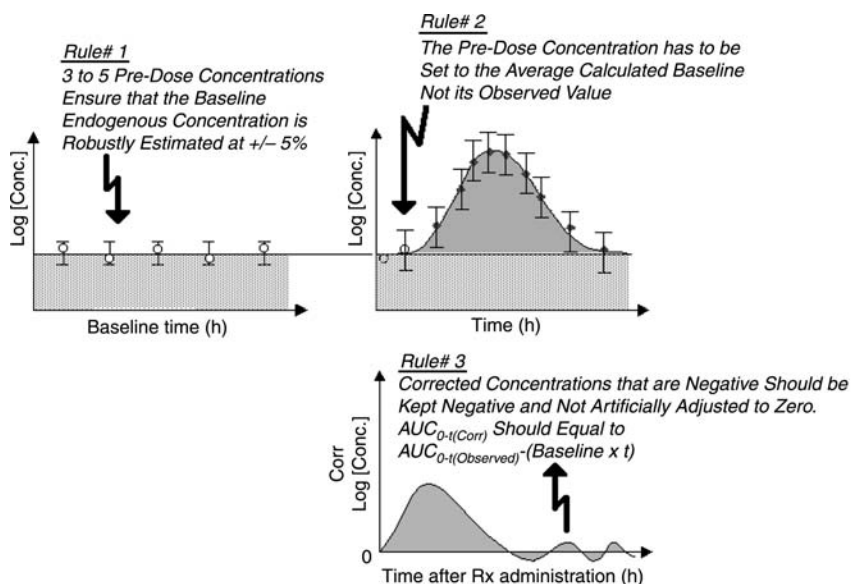


Figure 3 Three rules have to be adhered to for the correct calculation of the observed and maximum exposition due to exogenously administered compounds when the endogenous baseline is stable.

by lower concentrations in the evening. The baseline adjustment should then be subject-, sampling time- and period-specific. Ideally, the pre-dose sampling schedule should be identical to the post-dose sampling schedule over a 24-hour period and should cover the “ups” and “downs” of the circadian fluctuations. In that situation, the pre-dose baseline concentration at a specific time point must be subtracted from its corresponding post-dose concentration (Fig. 4). Negative values are included in the PK analysis for the same reasons as before. When values are missing because they were not collected in the clinic or were not reportable in the lab, adjustments must be made on a case by case basis. The adjusted PK parameters are calculated, after the data have been adjusted.

Unpredictably Unstable Baseline or Stable Concentrations

This is obviously the most difficult scenario. Two situations can occur, either separately or in conjunction. The baseline can be either unpredictably unstable or the concentrations can be unpredictably stable despite the administration of an endogenous drug. The first scenario means that the baseline cannot be predicted, while the alternate possibility may occur when despite administering different doses of the drug, the concentrations appear to be almost the same as the baseline concentrations. The conclusion is the same for both scenarios: Bioequivalence assessment will not be robust if the methods previously mentioned are used (e.g., assuming a predictably stable or unstable baseline). In the vast majority of cases, these phenomena are due to normal homeostatic processes in the body tightly regulating the concentrations of the endogenous substance. Two well-known examples are the systemic concentrations of potassium and those of levothyroxine (e.g., the

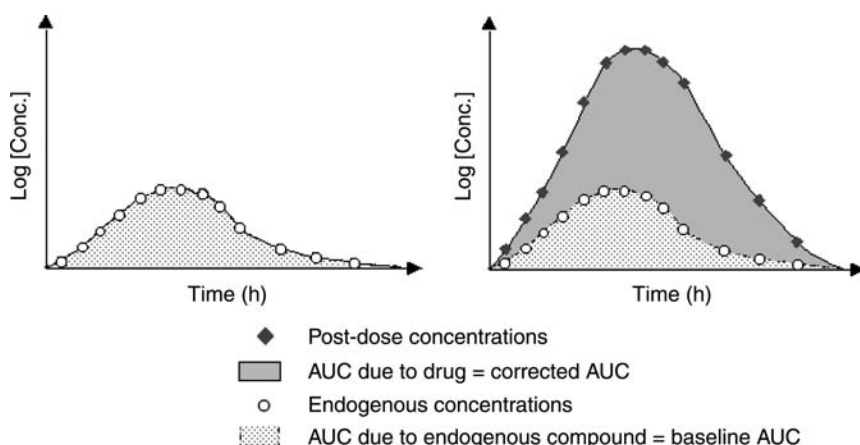


Figure 4 An example of a predictably unstable baseline adjustment.

synthetic form of L-Thyroxine. It will be denoted from now on in this chapter as “T4”). In general, systemic potassium levels are tightly regulated in healthy volunteers and high serum potassium concentrations can be seen either in hyperkalemic or hypokalemic individuals due to the redistribution of potassium from the intra to the extra-cellular compartments (9). This means that systemic potassium concentrations cannot help one discriminate between the relative bioavailability of two different formulations of potassium chloride. Indeed, a non-bioequivalent test formulation may always appear to be bioequivalent to the reference because the systemic concentrations of potassium will appear to be the same due to homeostasis factors. Regarding T4, concentrations are also highly regulated in healthy volunteers and possible interconversion between triiodothyronine (T3) and T4 may also help the body to further regulate the systemic levels of T4. Although this is a topic of great controversy, it is reasonable to think that it may not be robust to simply compare systemic exposure of T4 in healthy volunteers following the administration of test and reference formulations of T4 because of the normal feedback that will result in a change in the underlying baseline in order for the systemic concentrations of T4 to fluctuate as minimally as possible. A hypothetical graphical representation of what would happen in the case of systemic concentrations of potassium is shown in Figure 5.

There is no method for adjusting the baseline with these types of situations. What needs to be done instead, will be to find out why the

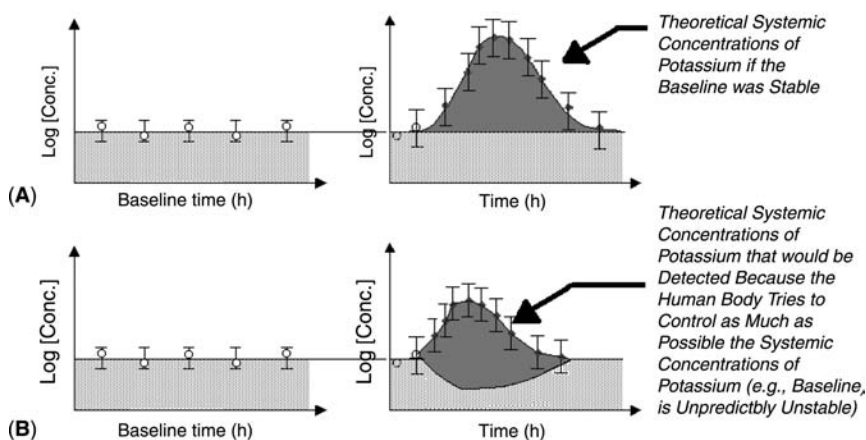


Figure 5 An example of an unpredictably unstable baseline: e.g., KCl. If the baseline was stable (A) then BE could be proven. However, since the baseline is not stable (B), BE cannot be reliably assessed using systemic potassium concentrations. Abbreviation: BE, bioequivalence.

baseline is unpredictably unstable and either control the cause or simply prove BE using a totally different method. In the case of potassium chloride, the solution will be to assess BE using urinary excreted amounts, whilst for the controversial levothyroxine case it may be preferable to assess BE in naturally or medically-induced athyroid patients.

STATISTICAL ANALYSIS

Statistical analysis of PK data in BE studies of endogenous compounds is performed on the baseline-adjusted PK parameters with an analysis of variance (ANOVA) using the now standard two one-sided *t*-test (10). The 90% confidence intervals (CIs) around the ratios of the test to reference products are calculated and need to lie completely within 80% to 125%.

Performing an ANOVA on the unadjusted response would not make much sense as it would substantially decrease the ability of the study to discriminate between the exposure of 2 different formulations of the same compounds due to the buffering effect of the baseline area. This is illustrated in Figure 6.

Regulators and scientists have debated for many years if an analysis of covariance (ANCOVA) would be more appropriate to use than an ANOVA, using the baseline as a covariate and either on the observed or adjusted PK parameters. An evaluation was therefore undertaken to establish the most appropriate statistical method for the assessment of the BE of two different formulations of an endogenous drug. Monte-Carlo simulations were performed and three different statistical methods were compared (11). The methods were compared on the basis of their Type I error and statistical power. The methods studied were the ANOVA performed on

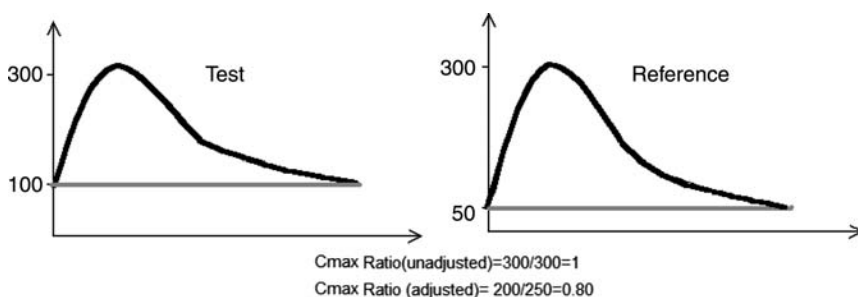


Figure 6 The statistical analysis has to be performed on adjusted PK parameters. Here an example showing a non-BE test compound to a reference one that would appear to be BE on the unadjusted data set simply due to the fact that the baseline is different between the different periods. *Abbreviations:* BE, bioequivalence; PK, pharmacokinetic.

baseline-adjusted parameters, the ANCOVA performed on the observed (unadjusted) parameters, and the ANCOVA performed on the baseline-adjusted parameters. One thousand two-way crossover studies were simulated under relative bioavailability (F_{rel}) conditions of Test/Reference ratios of either 1 (e.g., BE condition where the ratios lie completely between 0.8–1.25) or 1.3 (e.g., non-BE condition where the ratios lie outside the 0.8–1.25 acceptance range) using a one-compartment PK model. Simulations were performed with and without endogenous feedback. The endogenous feedback was simulated as depicted in Figure 5B, and represents an unpredictably unstable baseline. Table 1 depicts the results of the three different statistical methods for simulated studies of compounds that have no endogenous feedback while Table 2 shows the results of the three different statistical methods for simulated studies of compounds that have some endogenous feedback. Each table provides the percentage of studies passing the BE criteria for bioequivalent ($F_{\text{rel}} = 1$) and nonbioequivalent ($F_{\text{rel}} = 1.3$) products. The results are further considered in terms of the importance of the baseline, whether it is a small or a large component of the observed exposure, and also whether the baseline is statistically associated with the exposure or not.

Obviously, the objective of a chosen statistical test is to prove with a certain acceptable error that a drug product is truly bioequivalent (80%, or 1 minus the β error) or not (95%, or 1 minus the α error). The column showing %BE in Table 1 and 2 depict the percentage of studies where BE was concluded. Ideally, the results should be 100% and 0% for meeting the criteria of BE or not, respectively.

The results of these simulations confirm that the statistical analysis on the unadjusted PK parameters is inappropriate, even when an ANCOVA is used and the baseline is a statistically significant contributor of the overall PK profile. The ANCOVA on the unadjusted data should not be used to assess BE of endogenous compounds, due to its high Type I error (bold text in Table 1 and 2). This is consistent with what has been presented in Figure 6 above. Similar to performing the ANOVA on the unadjusted response, the ANCOVA performed on the unadjusted response would substantially decrease the ability of a study to discriminate between the exposure of 2 different formulations of the same compound due to the buffering effect of the baseline area. This finding became evident for compounds where the baseline represents a large portion of the observed exposure.

The results of these simulations indicated very little difference between the use of the ANOVA or the ANCOVA on the adjusted PK parameters. There was a trend for the ANCOVA on the baseline-adjusted response to perform better than the ANOVA when assessing BE for endogenous compounds when the percentage of baseline to unadjusted response was large (italics text in Table 1 and 2).

Table 1 Performance of ANOVA and ANCOVA to Assess the BE Between Two Formulations of the Same Drug when the Baseline Is Stable

$F_{\text{rel}} = 1$	%Baseline/ C_{avg}	8.9%			52.4%		
		%Base	%BE	%CV	%Base	%BE	%CV
	ANOVA, baseline-adjusted	—	93.9	14.1	—	79.6	17.1
	ANCOVA, unadjusted	6.4	94.5	12.8	67.8	100.0	7.7
	ANCOVA, baseline-adjusted	5.9	90.1	14.1	8.8	75.9	16.8
	Studies where baseline is significant						
	ANOVA, baseline-adjusted	—	94.6	13.7	—	72.3	18.3
	ANCOVA, unadjusted	100	95.1	9.5	100	100	7.2
	ANCOVA, baseline-adjusted	100	96.4	10.2	100	81.9	13.4
$F_{\text{rel}} = 1.3$	%Baseline/ C_{avg}	7.9%			49.2%		
		%Base	%BE	%CV	%Base	%BE	%CV
	ANOVA, baseline-adjusted	—	0.2	14.2	—	0.2	16.9
	ANCOVA, unadjusted	6.2	1.3	13.1	97.0	99.1	8.4
	ANCOVA, baseline-adjusted	4.4	0.1	14.3	12.2	0.1	16.7
	Studies where baseline is significant						
	ANOVA, baseline-adjusted	—	0.0	14.0	—	0.9	17.2
	ANCOVA, unadjusted	100	5.1	12.0	100	99.1	8.4
	ANCOVA, baseline-adjusted	100	0.0	12.9	100	0.0	15.7

%Baseline/ C_{avg} : proportion of the total exposure that is due to the baseline.

%Base: the percentage of studies where the baseline (used as a covariate in the ANCOVA model) was statistically significant at a 5% alpha level.

%BE: the percentage of studies where BE was concluded using the two-one-sided *t*-test approach. %CV: mean intra-subject CV.

Abbreviations: BE, bioequivalence; CV, coefficient of variation; ANOVA, analysis of variance; ANCOVA, analysis of covariance.

Table 2 Performance of ANOVA and ANCOVA to Assess the BE Between Two Formulations of the Same Drug when the Baseline Is Unpredictably Unstable (e.g., Negative Feedback is Present in the Biological System)

$F_{\text{rel}} = 1$	%Baseline/ C_{avg}	9.3%			61.5%		
		%Base	%BE	%CV	%Base	%BE	%CV
	ANOVA, baseline-adjusted	—	93.5	14.4	—	36.6	25.4
	ANCOVA, unadjusted	4.8	94.8	12.9	64.2	100	8.0
	ANCOVA, baseline-adjusted	5.8	89.6	14.3	18.1	37.7	23.3
	Studies where baseline is significant						
	ANOVA, baseline-adjusted	—	85.5	15.7	—	26.9	27.7
	ANCOVA, unadjusted	100	93.5	10.6	100	100	7.5
	ANCOVA, baseline-adjusted	100	89.1	11.7	100	47.4	20.4
$F_{\text{rel}} = 1.3$	%Baseline/ C_{avg}	8.3%			57.7%		
		%Base	%BE	%CV	%Base	%BE	%CV
	ANOVA, baseline-adjusted	—	0.2	14.8	—	0.0	23.9
	ANCOVA, unadjusted	6.2	1.1	13.5	94.2	95.9	8.9
	ANCOVA, baseline-adjusted	7.5	0.2	14.7	39.1	0.0	22.6
	Studies where baseline is significant						
	ANOVA, baseline-adjusted	—	0.0	14.9	—	0.0	25.0
	ANCOVA, unadjusted	100	0.0	12.3	100	95.9	8.8
	ANCOVA, baseline-adjusted	100	0.0	13.6	100	0.0	22.3

%Baseline/ C_{avg} : proportion of the total exposure that is due to the baseline.

%Base: the percentage of studies where the baseline (used as a covariate in the ANCOVA model) was statistically significant at a 5% alpha level.

% BE: the percentage of studies where BE was concluded using the two-one-sided *t*-test approach. %CV: mean intra-subject CV.

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; BE, bioequivalence; CV, coefficient of variation.

In summary, the statistical analysis should thus be performed on the adjusted PK parameters. Very little difference exists, if any, between performing a regular ANOVA analysis versus an ANCOVA. For simplicity purposes, the ANOVA may therefore be preferred.

CASE STUDIES

BE Assessment of Compounds with Unpredictably Unstable Baseline

Example is potassium chloride.

Background Information on Potassium Chloride

Potassium chloride (KCl) is an electrolyte replenisher and is indicated for the treatment or prevention of hypokalemia (potassium depletion) in certain patients. Normal doses of extended-release tablets range from 20 to 100 mEq or more of potassium daily, and are usually given in divided doses of no more than 20 mEq per dose (12).

As previously mentioned, the oral bioavailability of KCl preparations can be difficult to determine because the baseline will be unpredictably unstable. The potassium ion (K^+) is the principal intracellular cation of most body tissues, and its intracellular and plasma concentrations are maintained within narrow ranges by active ion transport. Serum or plasma potassium levels therefore do not reflect the potassium intake and are generally stable. Following the exogenous administration of potassium, the endogenous baseline concentration will therefore decrease in order for the overall systemic potassium levels to be as stable as possible. Thus, the bioavailability of an oral KCl preparation cannot be accurately determined by measuring post-dose plasma or serum K^+ concentrations. Rather, one simply needs to look at urinary excretion of potassium as an indicator of relative bioavailability. Urine is the major route of elimination for potassium, and under steady-state conditions in normal subjects, the amount of potassium supplied by the diet is directly proportional to the amount excreted in the urine (12,13). Studies of this type must be designed such that the potassium intake from all sources can be measured and controlled. The usual daily dietary requirement of potassium intake is 50 to 100 mEq per day, averaging 60 for females and 80 to 100 mEq for males. Anything administered above what is required will simply be excreted in the urine. Therefore, under steady-state conditions where potassium intake is monitored and maintained at a constant daily level, the amount of supplemental potassium chloride given will be excreted in the urine and will therefore be a direct reflection of its bioavailability. To ensure steady state conditions of potassium intake, the first four days of each period will be diet equilibration days to achieve and maintain a baseline level of urinary excretion of K^+ . This equilibration period should be followed by two days of baseline

measurements of excreted urinary potassium, and then by the administration of either the test or the reference KCl formulations.

Methodology

The current potassium studies were designed as randomized two-way crossover studies with each period having an equilibrium period (4 days), a baseline determination for excreted urinary potassium (2 days) and an actual collection period after a dose (2 days). Figure 7 depicts the chronological order of the study. The doses administered were 80 mEq (4 tablets) of potassium for each formulation of KCl.

A total of eight urinary samples were collected on each of the baseline determination days (Days 5, 6, 13 and 14) at the following times: 0 to 1, 1 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 16 and 16 to 24 hours. The same intervals were maintained for the first day after dosing (Days 7 and 15) with additional collection intervals at 24 to 36 and 36 to 48 hours after dosing (Days 8 and 16).

As previously mentioned, the only difference between two formulations of the same active ingredient will be in their rate and extent of exposure/bioavailability. The typical noncompartmental PK parameters calculated for BE studies using urinary outputs are the total amount excreted in the urine (A_e assessed over 24 ($A_{e\ 0-24}$) and 48 ($A_{e\ 0-48}$) hours for potassium chloride studies) and the maximum rate of excretion (R_{max}). The parameter $A_{e\ 0-48}$ provides an indication of the extent of exposure from time zero to the time at which urinary excretion will be back to baseline, while the maximum excretion rate will provide information on the rate of exposure. This is simply due to the

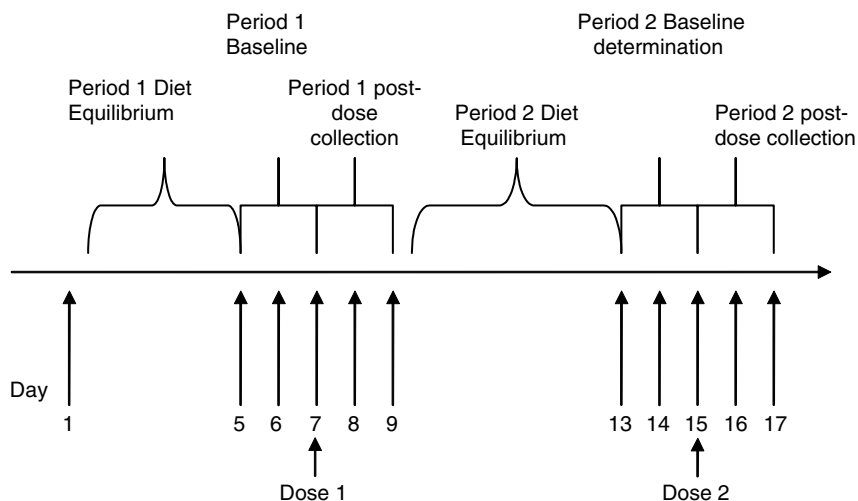


Figure 7 Schematic design of KCl bioequivalence study.

strong correlation in time between the maximum theoretical concentration in plasma and the maximum excretion rate of a drug that is excreted primarily in the urine. The total amount of potassium excreted unchanged in urine over an entire 24- or 48-hour period of sample collection ($A_{e\ 0-24}$, $A_{e\ 0-48}$) can be obtained by adding the amounts excreted over each collection interval. The urinary rate of excretion (R_{i-j}) of each collection interval ($i-j$) can be calculated by dividing the amount of drug excreted with the time over which it was collected. The $R_{\max}$ corresponds to the maximum rate of excretion of potassium and the time at which it is observed $T(R_{\max})$ is the midpoint of that particular corresponding interval. These parameters must be adjusted for the baseline excreted amounts. Corrections for baseline were subject, time interval and period specific. The excreted urinary amounts of potassium at each post-dose collection interval are adjusted by subtracting the mean of the excreted amounts from the corresponding time interval of the two baseline days. For example, the amount excreted during the 2 to 4 hour interval on Day 7 was corrected by subtracting the average amount collected during the 2 to 4 hour intervals on the baseline Days 5 and 6.

Statistical Analyses: The test to reference ratios for the point estimates as well as the 90% CIs for the key PK parameters previously mentioned that have been baseline corrected are required to be between 80% and 125%.

Consistent with the two one-sided test for BE (10), 90% CIs for the difference between drug formulation least-squares means (LSM) were calculated for the baseline corrected ln-transformed $A_{e\ 0-24}$, $A_{e\ 0-48}$ and $R_{\max}$ PK parameters. The 90% CIs for the test/reference ratios and their point estimates were obtained by exponentiation of the results of the ln-transformed PK parameters. The statistical analyses were performed using the SAS[®] GLM procedure.

Results and Discussion

Results presented in this case study are based on eight different potassium chloride BE studies, all with the same design discussed previously. Each study included between 30 and 35 subjects. As previously mentioned with potassium chloride, the plasma concentrations are unpredictably stable due to normal homeostatic processes in the body tightly regulating the concentrations. Therefore, BE can be demonstrated using excreted urinary amounts of potassium instead of plasma concentrations. The first step involves the analysis of the portion of the urinary output that is due to both the normal baseline endogenous excretion of potassium and the amount corresponding to the exogenously administered potassium. These two components of potassium excretion are represented graphically in [Figure 8](#) for the reference product. Excretion rates are presented as they are not affected by differences in interval durations, unlike amounts excreted, and therefore better illustrate the relatively stable nature of the baseline output depicted in blue.

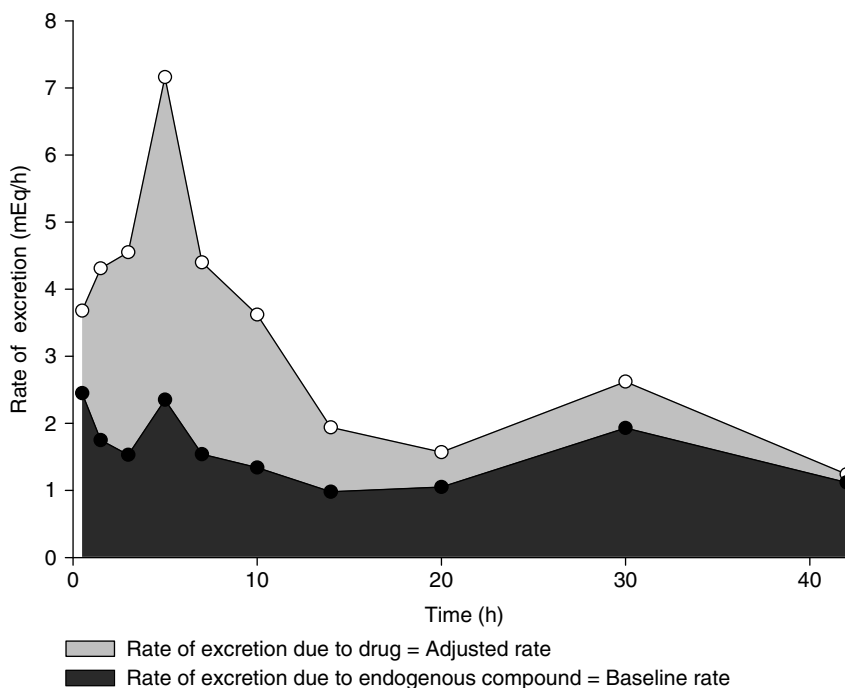


Figure 8 Observed urinary excretion rates of potassium following the administration of the KCl reference product.

The adjusted urinary rates of the two formulations (reference and test) that are due to the exogenous administration only (equivalent to the grey area in the previous figure) are presented in [Figure 9](#).

The cumulative amounts excreted from the two formulations are presented in [Figure 10](#).

It can be appreciated from [Figure 9](#) and [Figure 10](#) how the PK parameters $A_{e\ 0-24}$, $A_{e\ 0-48}$ and R_{max} are calculated. [Table 3](#) shows the BE assessment criteria results based on the average calculated PK parameters from all eight studies. All eight studies met the 80 to 125 BE assessment for $A_{e\ 0-24}$, but one and two studies failed the criteria for $A_{e\ 0-48}$ and R_{max} , respectively.

The study design presented in this chapter is in agreement with the Food and Drug Administration (FDA) guidances that have been published (14,15). Although the guidances were followed for these studies, we are not in agreement with the recommended amount of potassium in the diet that volunteers have to receive on the different baseline and treatment days. The FDA guidances suggests a slightly lower than normal intake of potassium of 50 to 60 mEq/day, a regular intake of 60 to 100 mEq/day would be preferable. Meeting regular daily requirements in potassium will

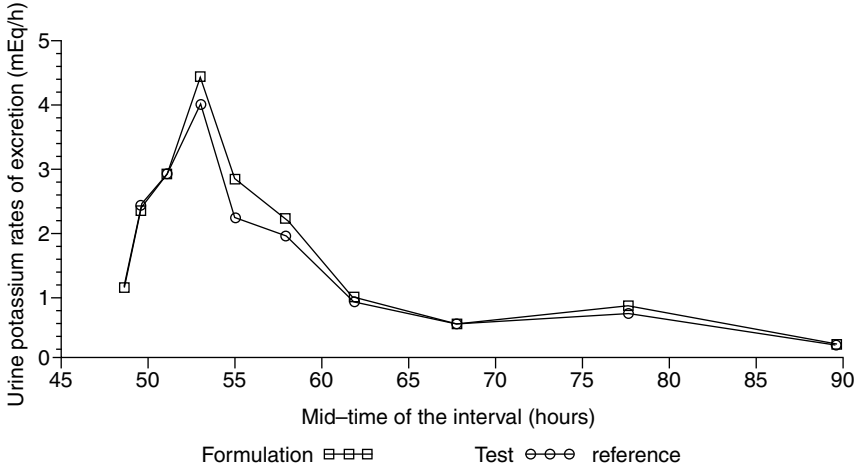


Figure 9 Adjusted urinary excretion rates of potassium following the administration of the reference KCl product.

obviate the build-up of any deficiencies in potassium levels in the body during the equilibration and baseline days. Otherwise, if potassium deficiencies build-up during the equilibrium and baseline phase, then these deficiencies will be partly replenished during the treatment days by the administration of the test or the reference product. Because of these

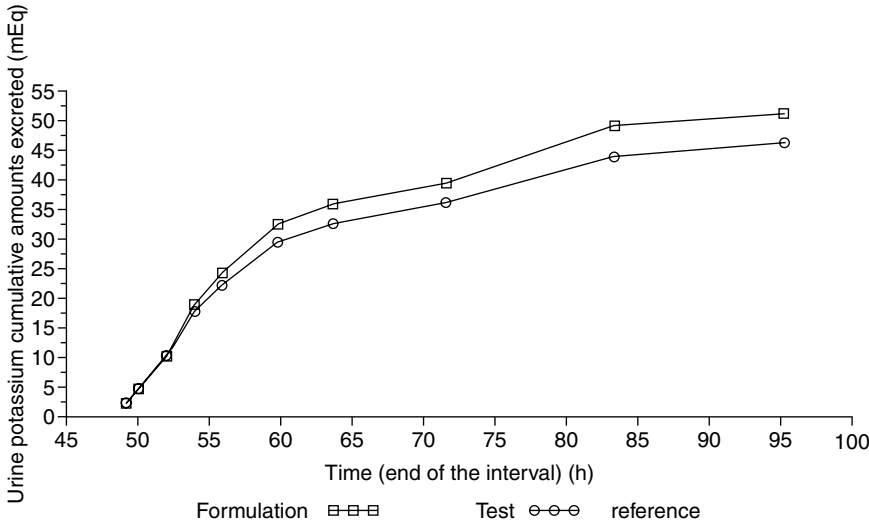


Figure 10 Adjusted cumulative excreted amounts of potassium following the administration of the test and reference KCl products.

Table 3 Average BE Assessment (ANOVA) for Potassium Chloride Adjusted Data

	Bioequivalence assessment		
	In $A_{e,0-24}$ ^a (mEq)	In $A_{e,0-48}$ ^a (mEq)	In R_{max} (mEq/h)
Average LSM: Unadjusted PK parameters (KDur [®] 80 mEq)	75.5	120.3	7.00
Average LSM: Adjusted PK parameters (KDur [®] 80 mEq)	38.7	46.8	4.84
Average ratios of LSM (90% CIs)	97.7% (90.7%–105.1%)	98.0% (88.7%–108.3%)	96.6% (87.3%–106.4%)

^a Antilog of mean presented.
Abbreviations: ANOVA, analysis of variance; BE, bioequivalence; CI, confidence Interval; PK, pharmacokinetic.

deficiencies, the baseline potassium excretion would not be correctly estimated since it would be underestimated. The study-results presented in [Table 1](#), using the lower recommended daily amounts of potassium presented in the FDA guidance, actually do suggest that administering a normal potassium diet might be better as only 50 mEq of the administered 80 mEq was excreted in the urine for the reference product. Since potassium’s bioavailability is expected to be virtually complete, then one would expect that virtually all of the administered 80 mEq dose should be excreted in the urine if no deficiency was present in volunteers. Although the current design appears to work well, it is recommended that consideration be given to administering a normal potassium diet (e.g., 60–100 mEq/day) to volunteers during the equilibration, baseline and treatment days instead of the low potassium diet (50–60 mEq). Therefore, when the potassium chloride test product or reference formulations are administered, volunteers could possibly excrete the entire bioavailable dose via the urine (instead of retaining part of it), thereby, allowing a better estimation of the relative bioavailability between the two formulations.

Conclusions

As discussed in section “Unpredictably Unstable Baseline or Stable Concentrations” of this chapter, systemic concentrations of potassium cannot be used to compare the relative bioavailability of two different KCl

formulations because of their unpredictably unstable nature resulting in a very tight homeostatic control of systemic potassium concentrations. Rather, the BE assessment can be performed using urinary excreted amounts of potassium as presented in this case study.

Bioequivalence Assessment on Compounds with Stable Baseline

Example is progesterone.

Background Information on Progesterone

Progesterone is a progestin hormone formed from steroid precursors located in the ovary, testis, adrenal cortex and the placenta. Progesterone secretion is stimulated by luteinizing hormone from the *corpus luteum* of the ovary during the latter half of the menstrual cycle. This steroid hormone is required for the implantation of the ovum and the maintenance of pregnancy. Secreted mainly during the luteal phase of the menstrual cycle with concentrations ranging from 3 to 25 ng/mL, progesterone concentrations are also observed in the follicular phase in postmenopausal women and also in men at levels below 1 ng/mL (16–18).

Administration of progesterone is indicated for the prevention of endometrial hyperplasia in non-hysterectomized postmenopausal women who are receiving conjugated estrogens preparations. Progesterone is also indicated for secondary amenorrhea. Progesterone should be given for the prevention of endometrial hyperplasia as a single 200 mg dose in the evening for 12 days sequentially per 28 day cycle to postmenopausal women with a uterus and who are receiving daily conjugated estrogens. Progesterone may also be given as a single dose of 400 mg in the evening for 10 days for secondary amenorrhea (16).

The oral bioavailability of progesterone is increased through micronization. Progesterone undergoes extensive first pass metabolism. Serum progesterone concentrations appear to increase in a predictable and dose proportional fashion following multiple administration over a dosing range of 100 to 300 mg daily. Progesterone is approximately 96% to 99% bound to serum proteins, primarily to serum albumin (50–54%) and transcortin[®] (43–48%) (16,18–20). Food increases the bioavailability of Prometrium[®] capsules relative to the fasting state when administered to postmenopausal women at a dose of 200 mg (16).

The metabolism of progesterone involves the participation of CYP enzymes. Progesterone is metabolized primarily by the liver, largely to pregnanediols and pregnanolones. The metabolites are then conjugated in the liver to glucuronides and sulfates and secreted in the bile or eliminated in the urine. The progesterone metabolites that are excreted in the bile may undergo enterohepatic recycling or may be eliminated in the faeces.

The most important metabolites that circulate in the blood are 17-hydroxyprogesterone (17-OHP), 11-desoxycorticosterone (DOC) and 20-dihydroprogesterone (20-DHP). Ten percent of progesterone is transformed to 20-DHP, which exhibits 25% to 50% of the progestational activity of progesterone (16,21).

Methodology

Progesterone is an example of a compound with stable systemic baseline levels in postmenopausal women. As previously described, it is therefore simple to calculate the exogenous portion of the drug administered. The information provided in this case study is based on five different progesterone studies.

They were designed as two-way randomized crossover studies in approximately 25 to 30 postmenopausal women with two different 200 mg progesterone formulations tested. Postmenopausal women were chosen in order to keep the baseline progesterone levels to a minimum and because of the stable nature of the observed measured progesterone concentrations (endogenous + exogenous) after exogenous administration of progesterone in that patient population. Ideally, baseline concentrations should be no higher than 5% of the $C_{\max}$, or the exposure due to the endogenous levels should be no greater than 10% to 20% of the observed exposure determined after an exogenous administration of the drug.

Baseline (endogenous) progesterone concentrations were measured over a 23-hour interval prior to dosing in Periods 1 and 2. Blood samples were collected at -23, -21, -19, -12 hours and at Hour 0 (pre-dose) for baseline determinations. In addition, samples were collected at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 30, and 36 hours post-dose. Unlike the previous case study, it was not necessary to schedule baseline concentrations at the same time as samples taken after the administration of progesterone. Here, five baseline concentrations were collected, minimizing the error in the baseline determination to a minimum (<5%). In addition, the timing of the baseline collections provided further support of the notion that the progesterone baseline was truly stable in that subject population.

Progesterone levels did not show a pattern throughout the day in any of the five studies examined. Therefore, the five baseline concentrations for each period were averaged. Concentrations from samples taken post-dose were adjusted by subtracting the mean baseline from every plasma concentration prior to the calculation of the PK parameters. This adjustment was subject and period specific.

The PK parameters of interest to determine whether the two different formulations were bioequivalent were the same as for any standard BE study based on systemic concentrations of administered drug. These are AUC_{0-t} , AUC_{inf} and $C_{\max}$. However, these PK parameters were calculated once the post-dose concentrations had been adjusted.

Statistical Analyses: With most typical BE studies, in order to meet regulatory guidelines for BE, the point estimates as well as the 90% CIs for the key PK parameters previously mentioned are required to be between 80% to 125%. However, for endogenous compounds, the ANOVA have to be performed on the ln-transformed baseline-adjusted AUC_{0-t} , AUC_{inf} and C_{max} parameters.

Consistent with the two one-sided test for BE (10), 90% CIs for the difference between drug formulation LSM were calculated for the baseline corrected ln-transformed AUC_{0-t} , AUC_{inf} and C_{max} parameters. The statistical analyses were performed using the SAS[®] GLM procedure. All ratios and 90% CIs were expressed as a percentage relative to the reference formulation.

Results and Discussion

For compounds displaying stable endogenous baseline levels, it is important to properly characterize this baseline level. Figure 11 illustrates the observed baseline concentrations for the reference product and its calculated

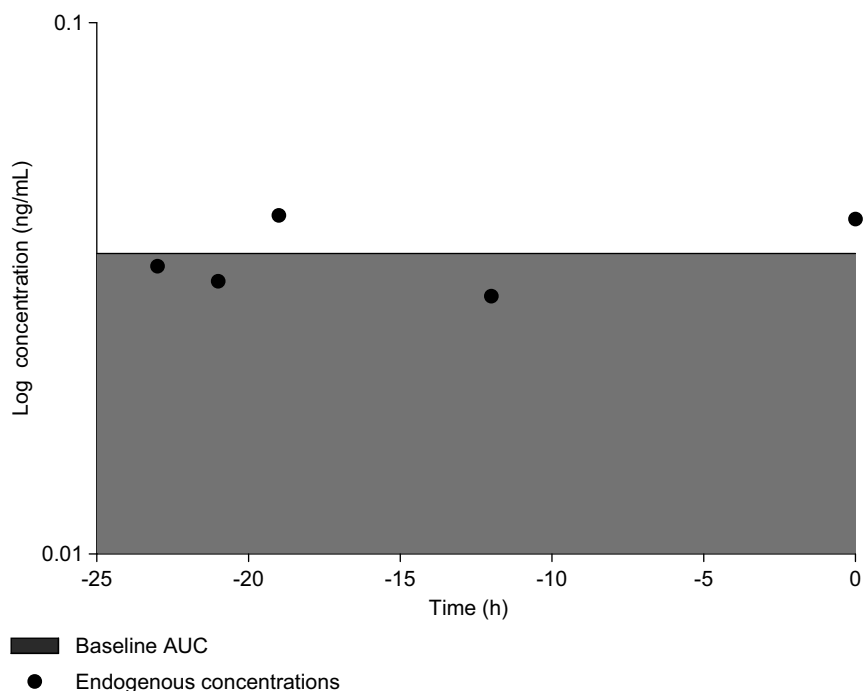


Figure 11 The stable baseline is assessed in a progesterone BE study by measuring and averaging five pre-dose concentrations. *Abbreviation:* BE, bioequivalence.

corresponding average level from one of the two studies. From this figure, it can be seen that the baseline concentrations were constant and using a mean baseline level was appropriate.

Following the determination of the baseline levels of progesterone, progesterone was administered exogenously. Progesterone concentrations up to 36 hours post-dose were measured. These measured concentrations are the summation of both the endogenous and exogenous components. Graphical representation of these concentrations for the same reference product presented in the previous figure can be seen in Figure 12. Baseline endogenous concentrations and exposure accounted for less than 1% and 2% of the observed progesterone $C_{\max}$ and exposure, respectively. This indicates that the adjusted concentrations could be robustly assessed and that the design of the study allowed for the appropriate determination of the BE of these two progesterone formulations administered exogenously. These concentrations for both the test and the reference products are presented in Figure 13.

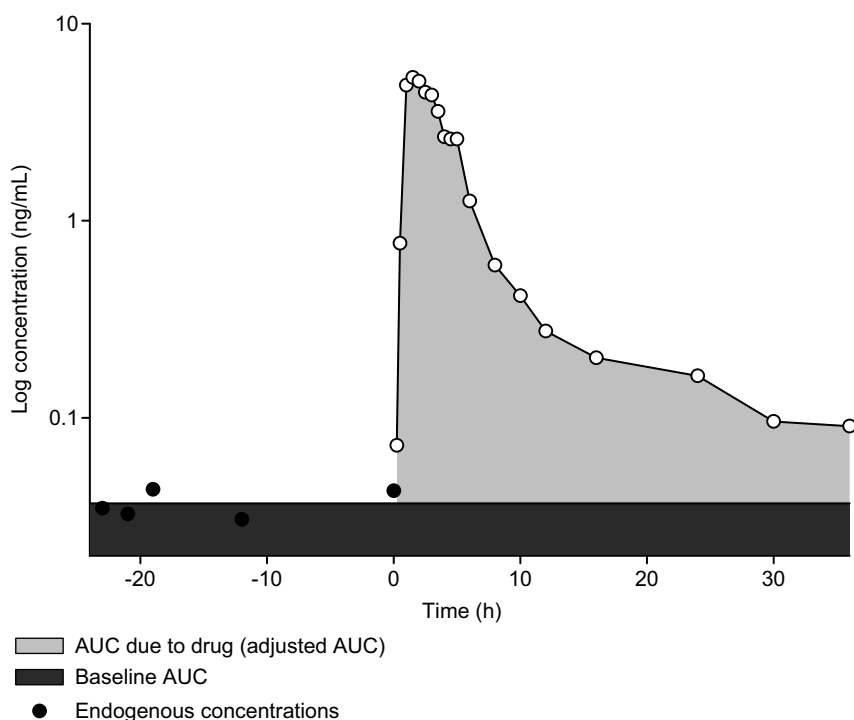


Figure 12 Observed (endogenous + exogenous) progesterone concentrations in a BE study. *Abbreviation:* BE, bioequivalence.

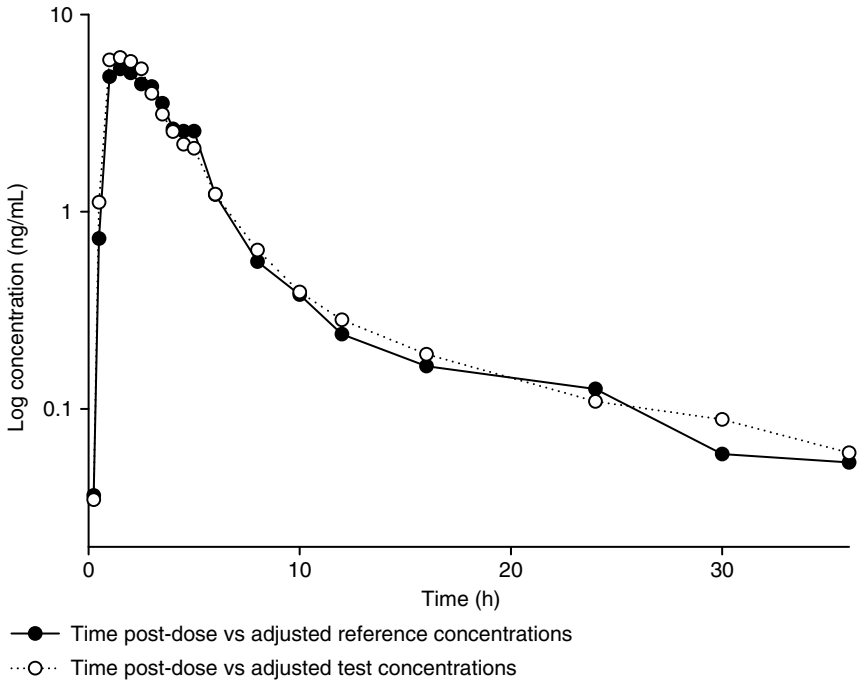


Figure 13 Adjusted post-dose progesterone concentrations in a BE study. *Abbreviation:* BE, bioequivalence.

Based on the adjusted concentrations, the PK parameters AUC_{0-t} , AUC_{inf} and C_{max} were calculated. Table 4 indicates the BE assessment criteria based on the average calculated PK parameters from both studies. Although the point estimates for all key PK parameters were within the accepted BE range, their 90% CIs were not.

Data in a Progesterone BE Study

Conclusions

The BE determination for a compound that displays a stable endogenous baseline after exogenous administration is a relatively straightforward

Table 4 Bioequivalence Assessment (ANOVA) for Progesterone Adjusted

	Bioequivalence assessment		
	In AUC_{0-1}	In AUC_{inf}	In C_{max}
Average ratio	93.6%	91.9%	86.5%
(90% CI)	(74.8–117.1%)	(70.4–120.0%)	(61.2–122.2%)

Abbreviations: BE, bioequivalence; ANOVA, analyses of variance; CI, coefficient of variation.

process, as presented in this case study for the drug progesterone. The baseline level has to be determined using a minimum of 3 to 5 concentrations to ensure that the resulting average concentration is as close as possible to the “true” baseline level. It is also important to ensure that the baseline levels are low compared to the total (endogenous and exogenous) levels measured, ensuring the robustness of the “adjusted” PK profiles for the test and reference formulations.

OVERALL CONCLUSIONS

A stepwise approach to assess the BE of two different formulations following administration of an endogenous drug has been presented. First, it is important to understand and assess the endogenous substance baseline. If it is predictably stable, then taking the average of 3 to 5 pre-dose concentrations will ensure that the calculated baseline should be within $\pm 5\%$ of its “true” value. A progesterone case study was presented to illustrate such an example. If the baseline is predictably not stable, then concentrations need to be taken during pre-dose baseline days at multiple time points to ensure that the diurnal variation has been correctly assessed. If the endogenous baseline is unpredictably unstable or stable, then an alternate study design should be pursued to overcome the hurdle. An example of this is the BE determination of two different formulations of potassium chloride where it was shown that the BE determination should be performed using excreted urinary amounts instead of plasma concentrations of potassium.

The second consideration relates to the PK analysis. It must be reflective of the exposure of what was exogenously administered so that the adjusted PK parameters must be calculated using adjusted concentrations (e.g., observed baseline concentrations). A few rules have been presented in this chapter to ensure that the BE assessment is correctly done. Finally, the statistical analysis must be performed using ANOVA on the adjusted PK parameters. An ANCOVA performed on the adjusted PK parameters and treating the baseline as a covariate can be done, but it does not appear to provide any additional benefit and is more complicated. Performing an ANCOVA on the un-adjusted PK parameters is clearly not appropriate.

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